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(54) Title: THERAPEUTIC AND DIAGNOSTIC METHODS AND COMPOSITIONS BASED ON NOTCH PROTEINS AND NUCLEIC ACIDS		
(57) Abstract The present invention relates to therapeutic and diagnostic methods and compositions based on Notch proteins and nucleic acids. Figure 17 displays the sequences of human Notch DNA and the encoded human Notch protein. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include Notch proteins and analogs and derivatives (including fragments) thereof, antibodies thereto, nucleic acids encoding the Notch proteins, analogs, or derivatives, Notch antisense nucleic acids, as well as topolythmic proteins and derivatives which bind to or otherwise interact with Notch proteins, their encoding nucleic acids or antibodies. In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state.		

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THERAPEUTIC AND DIAGNOSTIC METHODS AND COMPOSITIONS BASED ON NOTCH PROTEINS AND NUCLEIC ACIDS

5 This application is a continuation-in-part of copending application
Serial No. 08/083,590 filed June 25, 1993, which is a continuation-in-part of both
application Serial No. 07/955,012 filed September 30, 1992, now abandoned, and
copending application Serial No. 07/879,038 filed April 30, 1992, each of which
is incorporated by reference herein in its entirety.

10 This invention was made in part with government support under
grant numbers GM 29093 and NS 26084 awarded by the National Institutes of
Health. The government has certain rights in the invention.

1. INTRODUCTION

15 The present invention relates to therapeutic compositions
comprising Notch proteins, analogs and derivatives thereof, antibodies thereto,
nucleic acids encoding the Notch proteins, derivatives or analogs, Notch antisense
nucleic acids, and toporythmic proteins which bind to Notch and their nucleic
acids and antibodies. Therapeutic and diagnostic methods are also provided.

20 2. BACKGROUND OF THE INVENTION

2.1. THE NOTCH GENE AND PROTEIN

Null mutations in any one of the zygotic neurogenic loci -- Notch
(N), Delta (DI), mastermind (mam), Enhancer of Split (E(spl)), neuralized (neu),
25 and big brain (bib) -- result in hypertrophy of the nervous system at the expense of
ventral and lateral epidermal structures. This effect is due to the misrouting of
epidermal precursor cells into a neuronal pathway, and implies that neurogenic
gene function is necessary to divert cells within the neurogenic region from a
neuronal fate to an epithelial fate. Studies that assessed the effects of laser
30 ablation of specific embryonic neuroblasts in grasshoppers (Doe and Goodman
1985, Dev. Biol. 111, 206-219) have shown that cellular interactions between
neuroblasts and the surrounding accessory cells serve to inhibit these accessory

cells from adopting a neuroblast fate. Together, these genetic and developmental observations have led to the hypothesis that the protein products of the neurogenic loci function as components of a cellular interaction mechanism necessary for proper epidermal development (Artavanis-Tsakonas, 1988, Trends Genet. 4, 95-100).

Sequence analyses (Wharton et al., 1985, Cell 43, 567-581; Kidd et al., 1986, Mol. Cell. Biol. 6, 3094-3108; Vassin et al., 1987, EMBO J. 6, 3431-3440; Kopczynski et al., 1988, Genes Dev. 2, 1723-1735) have shown that two of the neurogenic loci, Notch and Delta, appear to encode transmembrane proteins that span the membrane a single time. The *Drosophila* Notch gene encodes a ~300 kd protein (we use "Notch" to denote this protein) with a large N-terminal extracellular domain that includes 36 epidermal growth factor (EGF)-like tandem repeats followed by three other cysteine-rich repeats, designated Notch/lin-12 repeats (Wharton et al., 1985, Cell 43, 567-581; Kidd et al., 1986, Mol. Cell Biol. 6, 3094-3108; Yochem et al., 1988, Nature 335, 547-550). The sequences of *Xenopus* (Coffman et al., 1990, Science 249:1438-1441) and a human Notch homolog termed *TAN-1* (Ellisen et al., 1991, Cell 66:649-661) have also been reported. Delta encodes a ~100 kd protein (we use "Delta" to denote DLZM, the protein product of the predominant zygotic and maternal transcripts; Kopczynski et al., 1988, Genes Dev. 2, 1723-1735) that has nine EGF-like repeats within its extracellular domain (Vassin et al., 1987, EMBO J. 6, 3431-3440; Kopczynski et al., 1988, Genes Dev. 2, 1723-1735). Although little is known about the functional significance of these repeats, the EGF-like motif has been found in a variety of proteins, including those involved in the blood clotting cascade (Furie and Furie, 1988, Cell 53, 505-518). In particular, this motif has been found in extracellular proteins such as the blood clotting factors IX and X (Rees et al., 1988, EMBO J. 7, 2053-2061; Furie and Furie, 1988, Cell 53, 505-518), in other *Drosophila* genes (Knust et al., 1987, EMBO J. 761-766; Rothberg et al., 1988, Cell 55, 1047-1059), and in some cell-surface receptor proteins, such as thrombomodulin (Suzuki et al., 1987, EMBO J. 6, 1891-1897) and LDL receptor (Sudhof et al., 1985, Science 228, 815-822). A protein binding site has

been mapped to the EGF repeat domain in thrombomodulin and urokinase (Kurosawa et al., 1988, J. Biol. Chem 263, 5993-5996; Appella et al., 1987, J. Biol. Chem. 262, 4437-4440).

5 An intriguing array of interactions between Notch and Delta mutations has been described (Vassin, et al., 1985, J. Neurogenet. 2, 291-308; Shepard et al., 1989, Genetics 122, 429-438; Xu et al., 1990, Genes Dev., 4, 464-475). A number of genetic studies (summarized in Alton et al., 1989, Dev. Genet. 10, 261-272) has indicated that the gene dosages of Notch and Delta in relation to one another are crucial for normal development. A 50% reduction in
10 the dose of Delta in a wild-type Notch background causes a broadening of the wing veins creating a "delta" at the base (Lindsley and Grell, 1968, Publication Number 627, Washington, D.C., Carnegie Institute of Washington). A similar phenotype is caused by a 50% increase in the dose of Notch in a wild-type Delta background (a "Confluens" phenotype; Welshons, 1965, Science 150, 1122-
15 1129). This Delta phenotype is partially suppressed by a reduction in the Notch dosage. Work has shown that lethal interactions between alleles that correlate with alterations in the EGF-like repeats in Notch can be rescued by reducing the dose of Delta (Xu et al., 1990, Genes Dev. 4, 464-475). Xu et al. (1990, Genes Dev. 4, 464-475) found that null mutations at either Delta or mam suppress lethal
20 interactions between heterozygous combinations of certain Notch alleles, known as the Abruptex (Ax) mutations. Ax alleles are associated with missense mutations within the EGF-like repeats of the Notch extracellular domain (Kelley et al., 1987, Cell 51, 539-548; Hartley et al., 1987, EMBO J. 6, 3407-3417).

Recent studies have shown that Notch and Delta, and Notch and
25 Serrate, directly interact on the molecular level (Fehon et al., 1990, Cell 61:523-534; Rebay et al., 1991, Cell 67:687-699).

Notch is expressed on axonal processes during the outgrowth of embryonic neurons (Johansen et al., 1989, J. Cell Biol. 109:2427-2440; Kidd et al., 1989, Genes Dev. 3:1113-1129; Fehon et al., 1991, J. Cell Biol.
30 113:657-669).

A study has shown that certain Ax alleles of Notch can severely alter axon pathfinding during sensory neural outgrowth in the imaginal discs, although it is not yet known whether aberrant Notch expression in the axon itself or the epithelium along which it grows is responsible for this defect (Palka et al.,
5 1990, Development 109, 167-175).

2.2. CANCER

A neoplasm, or tumor, is a neoplastic mass resulting from abnormal uncontrolled cell growth, which may cause swelling on the body
10 surface, and which can be benign or malignant. Benign tumors generally remain localized. Malignant tumors are collectively termed cancers. The term "malignant" generally means that the tumor can invade and destroy neighboring body structures and spread to distant sites to cause death (for review, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia,
15 pp. 68-122).

Effective treatment and prevention of cancer remains a long-felt need, and a major goal of biomedical research.

3. SUMMARY OF THE INVENTION

20 The present invention relates to therapeutic and diagnostic methods and compositions based on Notch proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Notch proteins and analogs and derivatives
25 (including fragments) thereof; antibodies thereto; nucleic acids encoding the Notch proteins, analogs, or derivatives; Notch antisense nucleic acids; as well as toporythmic proteins and derivatives which bind to or otherwise interact with Notch proteins, and their encoding nucleic acids and antibodies. In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous
30 condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a

Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect; disorders which can be thus treated can be identified by *in vitro* assays such as described in Section 5.1, *infra*. Such Antagonist Therapeutics include but are not limited to Notch antisense nucleic acids, anti-Notch neutralizing antibodies, and competitive inhibitors of Notch protein-protein interactions (*e.g.*, a protein comprising Notch ELR-11 and ELR-12 and derivatives thereof), all as detailed *infra*.

In another embodiment, Therapeutics which promote Notch function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect; disorders which can thus be treated can be identified by *in vitro* assays such as described in Section 5.1, *infra*. Such Agonist Therapeutics include but are not limited to Notch proteins and derivatives thereof comprising the intracellular domain, and proteins that interact with Notch (*e.g.*, a protein comprising a Delta sequence homologous to *Drosophila* Delta amino acids 1-230 (see Figure 1 and SEQ ID NO:2), or comprising a Serrate sequence homologous to *Drosophila* Serrate amino acids 79-282 (see Figure 5 and SEQ ID NO:4)).

Disorders of cell fate, in particular hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity of Notch protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of the proteins encoded by toporythmic genes which mediates binding to Notch proteins or adhesive fragments thereof. Toporythmic genes, as used herein, shall mean the genes Notch, Delta, and Serrate, as well as other members of the Delta/Serrate family which may be identified by virtue of sequence homology or genetic interaction, and in general, members of the "Notch cascade" or the "Notch group" of genes, which are identified by molecular interactions (*e.g.*,

binding *in vitro*) or genetic interactions (as detected phenotypically, *e.g.*, in *Drosophila*).

In another aspect, the invention is directed to human Notch proteins; in particular, that encoded by the hN homolog, and proteins comprising
5 the extracellular domain of the protein and subsequences thereof. Nucleic acids encoding the foregoing, and recombinant cells are also provided.

3.1. DEFINITIONS

As used herein, the following terms shall have the meanings
10 indicated:

	AA	=	amino acid
	EGF	=	epidermal growth factor
	ELR	=	EGF-like (homologous) repeat
	IC	=	intracellular
15	PCR	=	polymerase chain reaction

As used herein, underscoring the name of a gene shall indicate the gene, in contrast to its encoded protein product which is indicated by the name of the gene in the absence of any underscoring. For example, "Notch" shall mean the Notch gene, whereas "Notch" shall indicate the protein product of the Notch
20 gene.

4. DESCRIPTION OF THE FIGURES

Figure 1. Primary Nucleotide Sequence of the Delta cDNA D11 (SEQ ID NO:1) and Delta amino acid sequence (SEQ ID NO:2). The DNA
25 sequence of the 5'-3' strand of the D11 cDNA is shown, which contains a number of corrections in comparison to that presented in Kopczynski et al. (1988. Genes Dev. 2:1723-1735).

Figure 2. Notch Expression Constructs and the Deletion Mapping of the Delta/Serrate Binding Domain. S2 cells in log phase growth were
30 transiently transfected with the series of expression constructs shown; the drawings represent the predicted protein products of the various Notch deletion

mutants created. All expression constructs were derived from construct #1 pMtNMg. Transiently transfected cells were mixed with Delta expressing cells from the stably transformed line L49-6-7 or with transiently transfected Serrate expressing cells, induced with CuSO₄, incubated under aggregation conditions and then scored for their ability to aggregate using specific antisera and immunofluorescence microscopy. Aggregates were defined as clusters of four or more cells containing both Notch and Delta/Serrate expressing cells. The values given for % Aggregation refer to the percentage of all Notch expressing cells found in such clusters either with Delta (DI) (left column) or with Serrate (Ser) (right column). The various Notch deletion constructs are represented diagrammatically with splice lines indicating the ligation junctions. Each EGF repeat is denoted as a stippled rectangular box and numbers of the EGF repeats on either side of a ligation junction are noted. At the ligation junctions, partial EGF repeats produced by the various deletions are denoted by open boxes and closed brackets (for example see #23 Δ Cla+EGF(10-12)). Constructs #3-13 represent the ClaI deletion series. As diagrammed, four of the ClaI sites, in repeats 7, 9, 17 and 26, break the repeat in the middle, immediately after the third cysteine (denoted by open box repeats; see Figure 3 for further clarification), while the fifth and most 3' site breaks neatly between EGF repeats 30 and 31 (denoted by closed box repeat 31; again see Figure 3). In construct #15 split, EGF repeat 14 which carries the split point mutation, is drawn as a striped box. In construct #33 Δ Cla+XEGF(10-13), the *Xenopus* Notch derived EGF repeats are distinguished from *Drosophila* repeats by a different pattern of shading. SP, signal peptide; EGF, epidermal growth factor repeat; N, Notch/lin-12 repeat; TM, transmembrane domain; cdc10, cdc10/ankyrin repeats; PA, putative nucleotide binding consensus sequence; opa, polyglutamine stretch termed opa; DI, Delta; Ser, Serrate.

Figure 3. Detailed Structure of Notch Deletion Constructs #19-24: Both EGF Repeats 11 and 12 are Required for Notch-Delta Aggregation. EGF repeats 10-13 are diagrammed at the top showing the regular spacing of the six cysteine residues (C). PCR products generated for these constructs (names and

numbers as given in Figure 2) are represented by the heavy black lines and the exact endpoints are noted relative to the various EGF repeats. Ability to aggregate with Delta is recorded as (+) or (-) for each construct. The PCR fragments either break the EGF repeats in the middle, just after the third cysteine
 5 in the same place as four out of the five *Cla*I sites, or exactly in between two repeats in the same place as the most C-terminal *Cla*I site.

Figure 4. Comparison of Amino Acid Sequence of EGF Repeats 11 and 12 from *Drosophila* and *Xenopus* Notch. The amino acid sequence of EGF repeats 11 and 12 of *Drosophila* Notch (SEQ ID NO:14) (Wharton et al.,
 10 1985, Cell 43:567-581; Kidd et al., 1986, Mol. Cell Biol. 6:3094-3108) is aligned with that of the same two EGF repeats from *Xenopus* Notch (SEQ ID NO:15) (Coffman et al., 1990, Science 249:1438-1441). Identical amino acids are boxed. The six conserved cysteine residues of each EGF repeat and the Ca^{++} binding consensus residues (Rees et al., 1988, EMBO J. 7:2053-2061) are
 15 marked with an asterisk (*). The leucine to proline change found in the *Xenopus* PCR clone that failed to aggregate is noted underneath.

Figure 5. Nucleic Acid Sequence Homologies Between Serrate and Delta. A portion of the *Drosophila* Serrate nucleotide sequence (SEQ ID NO:3), with the encoded Serrate protein sequence (SEQ ID NO:4) written below
 20 (Fleming et al., 1990, Genes & Dev. 4:2188-2201 at 2193-94) is shown. The four regions showing high sequence homology with the *Drosophila* Delta sequence are numbered above the line and indicated by brackets. The total region of homology spans nucleotide numbers 627 through 1290 of the Serrate nucleotide sequence (numbering as in Figure 4 of Fleming et al., 1990, Genes & Dev.
 25 4:2188-2201).

Figure 6. Schematic Diagram of Human Notch Clones. A schematic diagram of human Notch is shown. Heavy bold-face lines below the diagram show that portion of the Notch sequence contained in each of the four cDNA clones. The location of the primers used in PCR, and their orientation,
 30 are indicated by arrows.

Figure 7. Human Notch Sequences Aligned with *Drosophila* Notch Sequence. Numbered vertical lines correspond to *Drosophila* Notch coordinates. Horizontal lines below each map show where clones lie relative to stretches of sequence (thick horizontal lines).

5 Figure 8. Nucleotide Sequences of Human Notch Contained in Plasmid cDNA Clone hN2k. Figure 8A: The DNA sequence (SEQ ID NO:5) of a portion of the human Notch insert is shown, starting at the EcoRI site at the 3' end, and proceeding in the 3' to 5' direction. Figure 8B: The DNA sequence (SEQ ID NO:6) of a portion of the human Notch insert is shown, starting at the
10 EcoRI site at the 5' end, and proceeding in the 5' to 3' direction. Figure 8C: The DNA sequence (SEQ ID NO:7) of a portion of the human Notch insert is shown, starting 3' of the sequence shown in Figure 8B, and proceeding in the 5' to 3' direction. The sequences shown are tentative, subject to confirmation by determination of overlapping sequences.

15 Figure 9. Nucleotide Sequences of Human Notch Contained in Plasmid cDNA clone hN4k. Figure 9A: The DNA sequence (SEQ ID NO:8) of a portion of the human Notch insert is shown, starting at the EcoRI site at the 5' end, and proceeding in the 5' to 3' direction. Figure 9B: The DNA sequence (SEQ ID NO:9) of a portion of the human Notch insert is shown, starting near
20 the 3' end, and proceeding in the 3' to 5' direction. The sequences shown are tentative, subject to confirmation by determination of overlapping sequences.

Figure 10. DNA (SEQ ID NO:10) and Amino Acid (SEQ ID NO:11) Sequences of Human Notch Contained in Plasmid cDNA Clone hN3k.

Figure 11. DNA (SEQ ID NO:12) and Amino Acid (SEQ ID
25 NO:13) Sequences of Human Notch Contained in Plasmid cDNA Clone hN5k.

Figure 12. Comparison of hN5k With Other Notch Homologs. Figure 12A. Schematic representation of *Drosophila* Notch. Indicated are the signal sequence (signal), the 36 EGF-like repeats, the three Notch/lin-12 repeats, the transmembrane domain (TM), the six CDC10 repeats, the OPA repeat, and
30 the PEST (proline, glutamic acid, serine, threonine)-rich region. Figure 12B. Alignment of the deduced amino acid sequence of hN5k with sequences of other

Notch homologs. Amino acids are numbered on the left side. The cdc10 and PEST-rich regions are both boxed, and individual cdc10 repeats are marked. Amino acids which are identical in three or more sequences are highlighted. The primers used to clone hN5k are indicated below the sequences from which they were designed. The nuclear localization sequence (NLS), casein kinase II (CKII), and cdc2 kinase (cdc2) sites of the putative CcN motif of the vertebrate Notch homologs are boxed. The possible bipartite nuclear targeting sequence (BNTS) and proximal phosphorylation sites of *Drosophila* Notch are also boxed.

Figure 13. Aligned amino acid sequences of Notch proteins of various species. humN: the human Notch protein encoded by the hN homolog (contained in part in plasmid hN5k) (SEQ ID NO:19). TAN-1: the human Notch protein encoded by the TAN-1 homolog (SEQ ID NO:20) (the sequence shown is derived partly from our own work and partly from the TAN-1 sequence as published by Ellisen et al., 1991, Cell 66:649-661); Xen N: *Xenopus* Notch protein (Coffman et al., 1990, Science 249:1438-1441). Dros N: *Drosophila* Notch protein (Wharton et al., 1985, Cell 43:567-581). Structural domains are indicated.

Figure 14. Immunocytochemical staining of breast cancer tissue from a human patient. Malignant breast tissue in a sample obtained from a human patient was embedded in a paraffin section, and subjected to immunocytochemical staining with anti-human Notch monoclonal antibody P4, directed against the TAN-1 protein. Non-malignant breast tissue exhibited much less staining (not shown).

Figure 15. Immunocytochemical staining of colon tissue from a human patient with colon cancer. A colon tissue sample obtained from a patient with colon cancer was embedded in a paraffin section, and subjected to immunocytochemical staining with anti-human Notch monoclonal antibody P1, directed against the hN-encoded protein. Areas of increased staining are those areas in which malignant cells are present, as determined by cell morphology.

Figure 16. Immunocytochemical staining of cervical tissue. Human tissue samples were obtained, containing cancer of the cervix (Fig. 16A) or normal cervical epithelium (Fig. 16B) from the same patient, embedded in a

paraffin section, and subjected to immunocytochemical staining with anti-human Notch monoclonal antibody directed against the TAN-1 protein. Areas containing malignant cells (as determined by morphology) exhibited increasing staining relative to non-malignant cells. Among non-malignant cells, connective tissue and the basal layer of the epithelium (containing stem cells) stained with the anti-Notch antibody.

Figure 17. DNA (SEQ ID NO:21) and encoded amino acid sequence (contained in SEQ ID NO:19) of human Notch homolog hN. The entire DNA coding sequence is presented (as well as noncoding sequence), with the exclusion of that encoding the initiator Met. The last 8 nucleotides shown (numbers 9716-9723) are vector, and not hN, sequences.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to therapeutic and diagnostic methods and compositions based on Notch proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Notch proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the Notch proteins, analogs, or derivatives; Notch antisense nucleic acids; as well as toporythmic proteins and derivatives and analogs thereof which bind to or otherwise interact with Notch proteins, and their encoding nucleic acids and antibodies. Also included are proteins and derivatives and analogs thereof which are capable of inhibiting the interactions of a Notch protein with another toporythmic protein (e.g. Delta, Serrate). In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state (e.g., metaplastic condition) into a neoplastic or a malignant state. In another specific embodiment, a Therapeutic of the invention is administered to treat a nervous system disorder, such as nerve injury or a degenerative disease. In yet another

specific embodiment, a Therapeutic of the invention is administered to promote tissue regeneration and repair for treatment of various conditions.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect; disorders which can be thus treated can be identified by *in vitro* assays such as described in Section 5.1, *infra*. Such Antagonist Therapeutics include but are not limited to Notch antisense nucleic acids, anti-Notch neutralizing antibodies, competitive inhibitors of Notch protein-protein interactions (*e.g.*, a protein comprising Notch ELR-11 and ELR-12), and molecules which interfere with notch intracellular function such as that mediated by the cdc10 repeats, as detailed *infra*.

In another embodiment, Therapeutics which promote Notch function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect; disorders which can thus be treated can be identified by *in vitro* assays such as described in Section 5.1, *infra*. Such Agonist Therapeutics include but are not limited to Notch proteins and derivatives thereof comprising the intracellular domain, Notch nucleic acids encoding the foregoing, and proteins comprising toporythmic protein domains that interact with Notch (*e.g.*, a protein comprising an extracellular domain of a Delta protein or a Delta sequence homologous to *Drosophila* Delta amino acids 1-230 (see Figure 1 and SEQ ID NO:2), or comprising a Serrate sequence homologous to *Drosophila* Serrate amino acids 79-282 (see Figure 5 and SEQ ID NO:4)).

Disorders of cell fate, in particular precancerous conditions such as metaplasia and dysplasia, and hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity of Notch protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of the proteins encoded by toporythmic genes which mediates binding to Notch proteins or adhesive fragments thereof. Toporythmic genes, as used herein, shall mean

the genes Notch, Delta, and Serrate, as well as other members of the Delta/Serrate family which may be identified by virtue of sequence homology or genetic interaction, and, more generally, members of the "Notch cascade" or the "Notch group" of genes, which are identified by molecular interactions (*e.g.*, binding *in vitro*) or genetic interactions (as detected phenotypically, *e.g.*, in *Drosophila*).

The invention further provides a human Notch protein encoded by the hN homolog, and proteins comprising the extracellular domain of the Notch protein and subsequences thereof. Nucleic acids encoding the foregoing, and recombinant cells are also provided.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the following subsections:

- (i) Therapeutic Uses;
- (ii) Prophylactic Uses;
- (iii) Demonstration of Therapeutic or Prophylactic Utility;
- (iv) Therapeutic/Prophylactic Administration and Compositions;
- (v) Antisense Regulation of Notch Expression;
- (vi) Diagnostic Utility;
- (vii) Notch Nucleic Acids;
- (viii) Recombinant Production of Protein Therapeutics;
- (ix) Derivatives and Analogs of Notch and Other Toporythmic Proteins;
- (x) Assays of Notch Proteins, Derivatives and Analogs; and
- (xi) Antibodies to Notch Proteins, Derivatives and Analogs.

5.1. THERAPEUTIC USES

As stated *supra*, the Antagonist Therapeutics of the invention are those Therapeutics which antagonize, or inhibit, a Notch function. Such Antagonist Therapeutics are most preferably identified by use of known convenient *in vitro* assays, *e.g.*, based on their ability to inhibit binding of Notch to other proteins (see Sections 6-8 herein), or inhibit any known Notch function

as assayed *in vitro*, although genetic assays (*e.g.*, in *Drosophila*) may also be employed. In a preferred embodiment, the Antagonist Therapeutic is a protein or derivative thereof comprising a functionally active fragment such as an adhesive fragment of Notch. In specific embodiments, such an Antagonist Therapeutic
5 may be those adhesive proteins encoded by the appropriate constructs described in Sections 6 and 7 *infra*, or proteins comprising the Notch extracellular region, in particular ELR-11 and ELR-12, or an antibody thereto, or an analog/competitive inhibitor of a Notch intracellular signal-transducing region, a nucleic acid capable
10 of expressing a Notch adhesive fragment, or a Notch antisense nucleic acid (see Section 5.5 herein). It should be noted that in certain instances, a Notch adhesive fragment (or possibly other presumed Antagonist Therapeutics) may alternatively act as an Agonist Therapeutic, depending on the developmental history of the tissue being exposed to the Therapeutic; preferably, suitable *in vitro* or *in vivo*
15 assays, as described *infra*, should be utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In another embodiment of the invention, a nucleic acid containing a portion of a Notch gene is used, as an Antagonist Therapeutic, to promote Notch inactivation by homologous recombination (Koller and Smithies, 1989,
20 Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

The Agonist Therapeutics of the invention, as described *supra*, promote Notch function. Such Agonist Therapeutics include but are not limited to proteins and derivatives comprising the portions of toporythmic proteins such
25 as Delta or Serrate that mediate binding to Notch, and nucleic acids encoding the foregoing (which can be administered to express their encoded products *in vivo*). In a specific embodiment, such a portion of Delta is *D. melanogaster* Delta amino acids 1-230 (SEQ ID NO:1) or a portion of a human Delta most homologous thereto. In another specific embodiment, such a portion of Serrate is *D.*
30 *melanogaster* Serrate amino acids 79-282 (SEQ ID NO:5), or a portion of a

human Serrate most homologous thereto. In other specific embodiments, such a portion of Delta or Serrate is the extracellular portion of such protein.

Further descriptions and sources of Therapeutics of the inventions are found in Sections 5.4 through 5.8 herein.

5 The Agonist and Antagonist Therapeutics of the invention have therapeutic utility for disorders of cell fate. The Agonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving an absence or decreased (relative to normal, or desired) levels of Notch function, for example, in patients where Notch protein is lacking,
10 genetically defective, biologically inactive or underactive, or underexpressed; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays (see *infra*) indicate the utility of Notch agonist administration. The absence or decreased levels in Notch function can be readily detected, *e.g.*, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for protein levels, structure
15 and/or activity of the expressed Notch protein. Many methods standard in the art can be thus employed, including but not limited to immunoassays to detect and/or visualize Notch protein (*e.g.*, Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.; see also those assays listed in Section 5.6, *infra*), and/or hybridization assays
20 to detect Notch expression by detecting and/or visualizing Notch mRNA (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.)

In vitro assays which can be used to determine whether administration of a specific Agonist Therapeutic or Antagonist Therapeutic is indicated, include *in vitro* cell culture assays in which a patient tissue sample is
25 grown in culture, and exposed to or otherwise administered a Therapeutic, and the effect of such Therapeutic upon the tissue sample is observed. In one embodiment, where the patient has a malignancy, a sample of cells from such malignancy is plated out or grown in culture, and the cells are then exposed to a Therapeutic. A Therapeutic which inhibits survival or growth of the malignant
30 cells (*e.g.*, by promoting terminal differentiation) is selected for therapeutic use *in vivo*. Many assays standard in the art can be used to assess such survival and/or

growth; for example, cell proliferation can be assayed by measuring ^3H -thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g.*, *fos*, *myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be
5 assessed visually based on changes in morphology, etc. In a specific aspect, the malignant cell cultures are separately exposed to (1) an Agonist Therapeutic, and (2) an Antagonist Therapeutic; the result of the assay can indicate which type of Therapeutic has therapeutic efficacy.

In another embodiment, a Therapeutic is indicated for use which
10 exhibits the desired effect, inhibition or promotion of cell growth, upon a patient cell sample from tissue having or suspected of having a hyper- or hypoproliferative disorder, respectively. Such hyper- or hypoproliferative disorders include but are not limited to those described in Sections 5.1.1 through 5.1.3 *infra*.

15 In another specific embodiment, a Therapeutic is indicated for use in treating nerve injury or a nervous system degenerative disorder (see Section 5.1.2) which exhibits *in vitro* promotion of nerve regeneration/neurite extension from nerve cells of the affected patient type.

In addition, administration of an Antagonist Therapeutic of the
20 invention is also indicated in diseases or disorders determined or known to involve a Notch dominant activated phenotype ("gain of function" mutations.) Administration of an Agonist Therapeutic is indicated in diseases or disorders determined or known to involve a Notch dominant negative phenotype ("loss of function" mutations). We have investigated the functions of various structural
25 domains of the Notch protein *in vivo*, by ectopically expressing a series of *Drosophila* Notch deletion mutants under the hsp70 heat-shock promoter, as well as eye-specific promoters. Two classes of dominant phenotypes were observed, one suggestive of *Notch* loss-of function mutations and the other of *Notch* gain-of-function mutations. Dominant "activated" phenotypes resulted from
30 overexpression of a protein lacking most extracellular sequences, while dominant "negative" phenotypes resulted from overexpression of a protein lacking most

intracellular sequences. Our results indicate that Notch functions as a receptor whose extracellular domain mediates ligand-binding, resulting in the transmission of developmental signals by the cytoplasmic domain. The phenotypes observed also suggested that the cdc10/ankyrin repeat region within the intracellular domain
5 plays an essential role in Notch mediated signal transduction events (intracellular function).

In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a Therapeutic has a desired effect upon such cell types.

10 In another embodiment, cells of a patient tissue sample suspected of being pre-neoplastic are similarly plated out or grown *in vitro*, and exposed to a Therapeutic. The Therapeutic which results in a cell phenotype that is more normal (i.e., less representative of a pre-neoplastic state, neoplastic state, malignant state, or transformed phenotype) is selected for therapeutic use. Many
15 assays standard in the art can be used to assess whether a pre-neoplastic state, neoplastic state, or a transformed or malignant phenotype, is present (see Section 5.2.1). For example, characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*) include a more rounded cell morphology, looser substratum attachment, loss of contact
20 inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton surface protein, etc. (see Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York pp. 436-446).

25 In other specific embodiments, the *in vitro* assays described *supra* can be carried out using a cell line, rather than a cell sample derived from the specific patient to be treated, in which the cell line is derived from or displays characteristic(s) associated with the malignant, neoplastic or pre-neoplastic disorder desired to be treated or prevented, or is derived from the neural or other
30 cell type upon which an effect is desired, according to the present invention.

The Antagonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving increased (relative to normal, or desired) levels of Notch function, for example, where the Notch protein is overexpressed or overactive; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays indicate the utility of Notch antagonist administration. The increased levels of Notch function can be readily detected by methods such as those described above, by quantifying protein and/or RNA. *In vitro* assays with cells of patient tissue sample or the appropriate cell line or cell type, to determine therapeutic utility, can be carried out as described above.

10

5.1.1. MALIGNANCIES

Malignant and pre-neoplastic conditions which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to those described below in Sections 5.1.1 and 5.2.1.

Malignancies and related disorders, cells of which type can be tested *in vitro* (and/or *in vivo*), and upon observing the appropriate assay result, treated according to the present invention, include but are not limited to those listed in Table 1 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

25

TABLE 1
MALIGNANCIES AND RELATED DISORDERS

30

Leukemia
 acute leukemia
 acute lymphocytic leukemia
 acute myelocytic leukemia
 myeloblastic
 promyelocytic
 myelomonocytic
 monocytic
 erythroleukemia

35

	chronic leukemia
	chronic myelocytic (granulocytic) leukemia
	chronic lymphocytic leukemia
	Polycythemia vera
	Lymphoma
5	Hodgkin's disease
	non-Hodgkin's disease
	Multiple myeloma
	Waldenström's macroglobulinemia
	Heavy chain disease
	Solid tumors
	sarcomas and carcinomas
10	fibrosarcoma
	myxosarcoma
	liposarcoma
	chondrosarcoma
	osteogenic sarcoma
	chordoma
	angiosarcoma
	endotheliosarcoma
15	lymphangiosarcoma
	lymphangioendotheliosarcoma
	synovioma
	mesothelioma
	Ewing's tumor
	leiomyosarcoma
	rhabdomyosarcoma
20	colon carcinoma
	pancreatic cancer
	breast cancer
	ovarian cancer
	prostate cancer
	squamous cell carcinoma
	basal cell carcinoma
	adenocarcinoma
25	sweat gland carcinoma
	sebaceous gland carcinoma
	papillary carcinoma
	papillary adenocarcinomas
	cystadenocarcinoma
	medullary carcinoma
	bronchogenic carcinoma
	renal cell carcinoma
30	hepatoma
	bile duct carcinoma
	choriocarcinoma
	seminoma

5 embryonal carcinoma
 Wilms' tumor
 cervical cancer
 testicular tumor
 lung carcinoma
 small cell lung carcinoma
 bladder carcinoma
 epithelial carcinoma
 glioma
 astrocytoma
 medulloblastoma
 craniopharyngioma
 ependymoma
 10 pinealoma
 hemangioblastoma
 acoustic neuroma
 oligodendroglioma
 meningioma
 melanoma
 neuroblastoma
 15 retinoblastoma

In specific embodiments, malignancy or dysproliferative changes
 (such as metaplasias and dysplasias) are treated or prevented in epithelial tissues
 such as those in the cervix, esophagus, and lung.

As detailed in the examples section 10.1 *infra*, malignancies of the
 breast, colon, and cervix exhibit increased expression of human Notch relative to
 such non-malignant tissue. Thus, in specific embodiments, malignancies of the
 breast, colon, or cervix are treated or prevented by administering an effective
 amount of an Antagonist Therapeutic of the invention. The presence of increased
 25 Notch expression in breast, colon, and cervical cancer suggests that many more
 cancerous conditions exhibit upregulated Notch. Thus, we envision that many
 more cancers, *e.g.*, seminoma, melanoma, and lung cancer, can be treated or
 prevented by administration of an Antagonist Therapeutic.

5.1.2. NERVOUS SYSTEM DISORDERS

- Nervous system disorders, involving cell types which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:
- (i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;
 - (ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;
 - (iii) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue;
 - (iv) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;
 - (v) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated

- 5 with Parkinson's disease, Alzheimer's disease,
Huntington's chorea, or amyotrophic lateral sclerosis;
- 10 (vi) lesions associated with nutritional diseases or disorders, in
which a portion of the nervous system is destroyed or
injured by a nutritional disorder or disorder of metabolism
including but not limited to, vitamin B12 deficiency, folic
acid deficiency, Wernicke disease, tobacco-alcohol
amblyopia, Marchiafava-Bignami disease (primary
degeneration of the corpus callosum), and alcoholic
cerebellar degeneration;
- 15 (vii) neurological lesions associated with systemic diseases
including but not limited to diabetes (diabetic neuropathy,
Bell's palsy), systemic lupus erythematosus, carcinoma, or
sarcoidosis;
- 20 (viii) lesions caused by toxic substances including alcohol, lead,
or particular neurotoxins; and
- (ix) demyelinated lesions in which a portion of the nervous
system is destroyed or injured by a demyelinating disease
including but not limited to multiple sclerosis, human
immunodeficiency virus-associated myelopathy, transverse
myelopathy or various etiologies, progressive multifocal
leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for
treatment of a nervous system disorder may be selected by testing for biological
25 activity in promoting the survival or differentiation of neurons (see also Section
5.1). For example, and not by way of limitation, Therapeutics which elicit any
of the following effects may be useful according to the invention:

- 30 (i) increased survival time of neurons in culture;
- (ii) increased sprouting of neurons in culture or *in vivo*;

- (iii) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or
- (iv) decreased symptoms of neuron dysfunction *in vivo*.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.

15 In a specific embodiments, motor neuron disorders that may be
treated according to the invention include but are not limited to disorders such as
infarction, infection, exposure to toxin, trauma, surgical damage, degenerative
disease or malignancy that may affect motor neurons as well as other components
of the nervous system, as well as disorders that selectively affect neurons such as
20 amyotrophic lateral sclerosis, and including but not limited to progressive spinal
muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and
juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-
Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary
Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

5.1.3. TISSUE REPAIR AND REGENERATION

In another embodiment of the invention, a Therapeutic of the invention is used for promotion of tissue regeneration and repair, including but not limited to treatment of benign dysproliferative disorders. Specific
30 embodiments are directed to treatment of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes), treatment of

keloid (hypertrophic scar) formation (disfiguring of the skin in which the scarring process interferes with normal renewal), psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination), and baldness (a condition in which terminally differentiated hair follicles (a tissue rich in Notch) fail to function properly).

5.2. PROPHYLACTIC USES

5.2.1. MALIGNANCIES

The Therapeutics of the invention can be administered to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 1. Such administration is indicated where the Therapeutic is shown in assays, as described *supra*, to have utility for treatment or prevention of such disorder. Such prophylactic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can
5 indicate the desirability of prophylactic/therapeutic administration of a Therapeutic of the invention. As mentioned *supra*, such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens,
10 disappearance of the 250,000 dalton cell surface protein, etc. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of
15 prophylactic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) is indicative of the desirability of prophylactic intervention.

In other embodiments, a patient which exhibits one or more of the
20 following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy
25 (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome,
30 neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma

pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; *see* Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

5 In another specific embodiment, an Antagonist Therapeutic of the invention is administered to a human patient to prevent progression to breast, colon, or cervical cancer.

5.2.2. OTHER DISORDERS

10 In other embodiments, a Therapeutic of the invention can be administered to prevent a nervous system disorder described in Section 5.1.2, or other disorder (*e.g.*, liver cirrhosis, psoriasis, keloids, baldness) described in Section 5.1.3.

15 5.3. DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY

The Therapeutics of the invention can be tested *in vivo* for the desired therapeutic or prophylactic activity. For example, such compounds can be tested in suitable animal model systems prior to testing in humans, including
20 but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used.

25 5.4. THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS

The invention provides methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a Therapeutic of the invention. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.
30

Various delivery systems are known and can be used to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles,

microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, 5 intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be 10 systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

15 In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a 20 suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

25 In a specific embodiment, administration of a Therapeutic into a Notch-expressing cell is accomplished by linkage of the Therapeutic to a Delta (or other toporythmic) protein or portion thereof capable of mediating binding to Notch. Contact of a Notch-expressing cell with the linked Therapeutic results in binding of the linked Therapeutic via its Delta portion to Notch on the surface of 30 the cell, followed by uptake of the linked Therapeutic into the Notch-expressing cell.

In a specific embodiment wherein an analog of a Notch intracellular signal-transducing domain is employed as a Therapeutic, such that it can inhibit Notch signal transduction, the analog is preferably delivered intracellularly (*e.g.*, by expression from a nucleic acid vector, or by linkage to a Delta protein capable of binding to Notch followed by binding and internalization, or by receptor-mediated mechanisms).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

In specific embodiments directed to treatment or prevention of particular disorders, preferably the following forms of administration are used:

	<u>Disorder</u>	<u>Preferred Forms of Administration</u>
	Cervical cancer	Topical
	Gastrointestinal cancer	Oral; intravenous
	Lung cancer	Inhaled; intravenous
25	Leukemia	Intravenous; extracorporeal
	Metastatic carcinomas	Intravenous; oral
	Brain cancer	Targeted; intravenous; intrathecal
	Liver cirrhosis	Oral; intravenous
	Psoriasis	Topical
30	Keloids	Topical
	Baldness	Topical

Spinal cord injury	Targeted; intravenous; intrathecal
Parkinson's disease	Targeted; intravenous; intrathecal
Motor neuron disease	Targeted; intravenous; intrathecal
Alzheimer's disease	Targeted; intravenous; intrathecal

5

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol,
 10 and combinations thereof. The carrier and composition can be sterile. The formulation should suit the mode of administration.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release
 15 formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

20 In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local
 25 anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by
 30 infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by

injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free
5 amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be
10 effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of
15 the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body
20 weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

25 The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products,
30 which notice reflects approval by the agency of manufacture, use or sale for human administration.

5.5. ANTISENSE REGULATION OF NOTCH EXPRESSION

The present invention provides the therapeutic or prophylactic use of nucleic acids of at least six nucleotides that are antisense to a gene or cDNA encoding Notch or a portion thereof. "Antisense" as used herein refers to a nucleic acid capable of hybridizing to a portion of a Notch RNA (preferably mRNA) by virtue of some sequence complementarity. Such antisense nucleic acids have utility as Antagonist Therapeutics of the invention, and can be used in the treatment or prevention of disorders as described *supra* in Section 5.1 and its subsections.

The antisense nucleic acids of the invention can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA or a modification or derivative thereof, which can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences.

In a specific embodiment, the Notch antisense nucleic acids provided by the instant invention can be used for the treatment of tumors or other disorders, the cells of which tumor type or disorder can be demonstrated (*in vitro* or *in vivo*) to express the Notch gene. Such demonstration can be by detection of Notch RNA or of Notch protein.

The invention further provides pharmaceutical compositions comprising an effective amount of the Notch antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described *supra* in Section 5.4. Methods for treatment and prevention of disorders (such as those described in Sections 5.1 and 5.2) comprising administering the pharmaceutical compositions of the invention are also provided.

In another embodiment, the invention is directed to methods for inhibiting the expression of a Notch nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an antisense Notch nucleic acid of the invention.

In another embodiment, the identification of cells expressing functional Notch receptors can be carried out by observing the ability of Notch to "rescue" such cells from the cytotoxic effects of a Notch antisense nucleic acid.

In an alternative embodiment of the invention, nucleic acids antisense to a nucleic acid encoding a ("adhesive") toporythmic protein or fragment that binds to Notch, are envisioned as Therapeutics.

Notch antisense nucleic acids and their uses are described in detail
5 below.

5.5.1. NOTCH ANTISENSE NUCLEIC ACIDS

The Notch antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 50 oligonucleotides).
10 In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate
15 backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or blood-brain barrier (see, *e.g.*,
20 PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549).

In a preferred aspect of the invention, a Notch antisense oligonucleotide is provided, preferably of single-stranded DNA. In a most
25 preferred aspect, such an oligonucleotide comprises a sequence antisense to the sequence encoding ELR 11 and ELR 12 of Notch, most preferably, of human Notch. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The Notch antisense oligonucleotide may comprise at least one
30 modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine,

xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil,
 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil,
 dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine,
 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine,
 5 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine,
 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-
 2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil,
 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v),
 wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil,
 10 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester,
 uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil. 3-(3-amino-3-N-2-
 carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In another embodiment, the oligonucleotide comprises at least one
 modified sugar moiety selected from the group including but not limited to
 15 arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least
 one modified phosphate backbone selected from the group consisting of a
 phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a
 phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl
 20 phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the oligonucleotide is an α -anomeric
 oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded
 hybrids with complementary RNA in which, contrary to the usual β -units, the
 strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res.
 25 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, *e.g.*,
 a peptide, hybridization triggered cross-linking agent, transport agent,
 hybridization-triggered cleavage agent, etc.

Oligonucleotides of the invention may be synthesized by standard
 30 methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as
 are commercially available from Biosearch, Applied Biosystems, etc.). As

examples, phosphorothioate oligos may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligos can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

5 In a specific embodiment, the Notch antisense oligonucleotide comprises catalytic RNA, or a ribozyme (see, *e.g.*, PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225). In another embodiment, the oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA
10 analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

In an alternative embodiment, the Notch antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed,
15 producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the Notch antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art.
20 Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the Notch antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region
25 (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), etc.

30 The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a Notch gene,

preferably a human Notch gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of
5 double-stranded Notch antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a Notch RNA it may contain and still form a stable duplex (or
10 triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

15 5.5.2. THERAPEUTIC UTILITY OF NOTCH ANTISENSE NUCLEIC ACIDS

The Notch antisense nucleic acids can be used to treat (or prevent) malignancies, of a cell type which has been shown to express Notch RNA. Malignant, neoplastic, and pre-neoplastic cells which can be tested for such expression include but are not limited to those described *supra* in Sections 5.1.1
20 and 5.2.1. In a preferred embodiment, a single-stranded DNA antisense Notch oligonucleotide is used.

Malignant (particularly, tumor) cell types which express Notch RNA can be identified by various methods known in the art. Such methods include but are not limited to hybridization with a Notch-specific nucleic acid
25 (e.g. by Northern hybridization, dot blot hybridization, *in situ* hybridization), observing the ability of RNA from the cell type to be translated *in vitro* into Notch, etc. In a preferred aspect, primary tumor tissue from a patient can be assayed for Notch expression prior to treatment.

Pharmaceutical compositions of the invention (see Section 5.1.4),
30 comprising an effective amount of a Notch antisense nucleic acid in a pharmaceutically acceptable carrier, can be administered to a patient having a malignancy which is of a type that expresses Notch RNA.

The amount of Notch antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity of the tumor
5 type to be treated *in vitro*, and then in useful animal model systems prior to testing and use in humans.

In a specific embodiment, pharmaceutical compositions comprising Notch antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, it may be useful to use
10 such compositions to achieve sustained release of the Notch antisense nucleic acids. In a specific embodiment, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable tumor antigens (Leonetti et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2448-2451; Renneisen et al., 1990, J. Biol. Chem. 265:16337-16342).

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5.6. DIAGNOSTIC UTILITY

Notch proteins, analogues, derivatives, and subsequences thereof, Notch nucleic acids (and sequences complementary thereto), anti-Notch antibodies, and other toporythmic proteins and derivatives and analogs thereof
20 which interact with Notch proteins, and inhibitors of North-toporythmic protein interactions, have uses in diagnostics. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting Notch expression, or monitor the treatment thereof. In particular, such an immunoassay is carried out by a method
25 comprising contacting a sample derived from a patient with an anti-Notch antibody under conditions such that immunospecific binding can occur, and detecting or measuring the amount of any immunospecific binding by the antibody. In a specific embodiment, antibody to Notch can be used to assay in a patient tissue or serum sample for the presence of Notch where an aberrant level
30 of Notch is an indication of a diseased condition.

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The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel
5 diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few.

Notch genes and related nucleic acid sequences and subsequences, including complementary sequences, and other toporythmic gene sequences, can
10 also be used in hybridization assays. Notch nucleic acid sequences, or subsequences thereof comprising about at least 8 nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in Notch expression and/or activity as described *supra*. In
15 particular, such a hybridization assay is carried out by a method comprising contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to Notch DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

As detailed in examples section 10.1 *infra*, increased Notch
20 expression occurs in human breast, colon, and cervical cancer. Accordingly, in specific embodiments, human breast, colon, or cervical cancer or premalignant changes in such tissues is diagnosed by detecting increased Notch expression (or amount) in patient samples relative to the level of Notch expression (or amount) in an analogous non-malignant, or non-premalignant, as the case may be, sample
25 (from the patient or another person, as determined experimentally or as is known as a standard level in such samples).

In one embodiment, the Notch protein (or derivative having Notch antigenicity) that is detected or measured is on the cell surface. In another embodiment, the Notch protein (or derivative) is a cell free soluble molecule
30 (*e.g.*, as measured in a blood or serum sample) or is intracellular. Without intending to be bound mechanistically, Applicants believe that cell free Notch may

result from secretion or shedding from the cell surface. In yet another embodiment, soluble, cell-surface, and intracellular amounts of Notch protein or derivative are detected or measured.

5

5.7. NOTCH NUCLEIC ACIDS

Therapeutics of the invention which are Notch nucleic acids or Notch antisense nucleic acids, as well as nucleic acids encoding protein Therapeutics, include those described below, which can be obtained by methods known in the art, and in particular, as described below.

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In particular aspects, the invention provides amino acid sequences of Notch, preferably human Notch, and fragments and derivatives thereof which comprise an antigenic determinant (i.e., can be recognized by an antibody) or which are functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" material as used herein refers to that material displaying one or more known functional activities associated with the full-length (wild-type) Notch protein product, *e.g.*, binding to Delta, binding to Serrate, binding to any other Notch ligand, antigenicity (binding to an anti-Notch antibody), etc.

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In specific embodiments, the invention provides fragments of a Notch protein consisting of at least 40 amino acids, or of at least 75 amino acids. In other embodiments, the proteins comprise or consist essentially of the intracellular domain, transmembrane region, extracellular domain, cdc10 region, Notch/lin-12 repeats, or the EGF-homologous repeats, or any combination of the foregoing, of a Notch protein. Fragments, or proteins comprising fragments, lacking some or all of the EGF-homologous repeats of Notch are also provided. Nucleic acids encoding the foregoing are provided.

20

25

In other specific embodiments, the invention provides nucleotide sequences and subsequences of Notch, preferably human Notch, consisting of at least 25 nucleotides, at least 50 nucleotides, or at least 150 nucleotides. Nucleic acids encoding the proteins and protein fragments described above are provided, as well as nucleic acids complementary to and capable of hybridizing to such

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nucleic acids. In one embodiment, such a complementary sequence may be complementary to a Notch cDNA sequence of at least 25 nucleotides, or of at least 100 nucleotides. In a preferred aspect, the invention utilizes cDNA sequences encoding human Notch or a portion thereof. In a specific embodiment, such sequences of the human Notch gene or cDNA are as contained in plasmids hN3k, hN4k, or hN5k (see Section 9, *infra*) or in the gene corresponding thereto; such a human Notch protein sequence can be as shown in Figures 10 (SEQ ID NO:11) or 11 (SEQ ID NO:13). In other embodiments, the Notch nucleic acid and/or its encoded protein has at least a portion of the sequence shown in one of the following publications: Wharton et al., 1985, Cell 43:567-581 (*Drosophila* Notch); Kidd et al., 1986, Mol. Cell. Biol. 6:3094-3108 (*Drosophila* Notch); Coffman et al., 1990, Science 249:1438-1441 (*Xenopus* Notch); Ellisen et al., 1991, Cell 66:649-661 (a human Notch). In another aspect, the sequences of human Notch are those encoding the human Notch amino acid sequences or a portion thereof as shown in Figure 13. In a particular aspect, the human Notch sequences are those of the hN homolog (represented in part by plasmid hN5k) or the TAN-1 homolog.

In one embodiment of the invention, the invention is directed to the full-length human Notch protein encoded by the hN homolog as depicted in Figure 13, both containing the signal sequence (*i.e.*, the precursor protein; amino acids 1-2169) and lacking the signal sequence (*i.e.*, the mature protein; amino acids ~26-2169), as well as portions of the foregoing (*e.g.*, the extracellular domain, EGF homologous repeat region, EGF-like repeats 11 and 12, cdc-10/ankyrin repeats, etc.) and proteins comprising the foregoing, as well as nucleic acids encoding the foregoing.

As is readily apparent, as used herein, a "nucleic acid encoding a fragment or portion of a Notch protein" shall be construed as referring to a nucleic acid encoding only the recited fragment or portion of the Notch protein and not other portions of the Notch protein.

In a preferred, but not limiting, aspect of the invention, a human Notch DNA sequence can be cloned and sequenced by the method described in Section 9, *infra*.

5 In another preferred aspect, PCR is used to amplify the desired sequence in the library, prior to selection. For example, oligonucleotide primers representing part of the adhesive domains encoded by a homologue of the desired gene can be used as primers in PCR.

The above-methods are not meant to limit the following general description of methods by which clones of Notch may be obtained.

10 Any eukaryotic cell can potentially serve as the nucleic acid source for the molecular cloning of the Notch gene. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired human cell (see, for example
15 Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 2d. Ed., Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA
20 will contain only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively,
25 one may use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

30 Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene may be accomplished in a

number of ways. For example, if an amount of a portion of a Notch (of any species) gene or its specific RNA, or a fragment thereof *e.g.*, the adhesive domain, is available and can be purified and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe
5 (Benton, W. and Davis, R., 1977, Science 196, 180; Grunstein, M. And Hogness, D., 1975, Proc. Natl. Acad. Sci. U.S.A. 72, 3961). Those DNA fragments with substantial homology to the probe will hybridize. It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known
10 restriction map if such is available. Further selection can be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein
15 that, *e.g.*, has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, *in vitro* aggregation activity ("adhesiveness") or antigenic properties as known for Notch. If an antibody to Notch is available, the Notch protein may be identified by binding of labeled antibody to the putatively Notch synthesizing clones. in an ELISA (enzyme-linked
20 immunosorbent assay)-type procedure.

The Notch gene can also be identified by mRNA selection by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified Notch DNA of another species
25 (*e.g.*, *Drosophila*). Immunoprecipitation analysis or functional assays (*e.g.*, aggregation ability *in vitro*; see examples *infra*) of the *in vitro* translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated
30 from cells to immobilized antibodies specifically directed against Notch or Delta protein. A radiolabelled Notch cDNA can be synthesized using the selected

mRNA (from the adsorbed polysomes) as a template. The radiolabelled mRNA or cDNA may then be used as a probe to identify the Notch DNA fragments from among other genomic DNA fragments.

5 Alternatives to isolating the Notch genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the Notch gene. For example, RNA for cDNA cloning of the Notch gene can be isolated from cells which express Notch. Other methods are possible and within the scope of the invention.

10 The identified and isolated gene can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda
15 derivatives, or plasmids such as PBR322 or pUC plasmid derivatives. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be
20 enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and Notch or Delta gene may be modified by homopolymeric
25 tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach.
30 Enrichment for the desired gene, for example, by size fractionization, can be done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated Notch gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when
5 necessary, retrieving the inserted gene from the isolated recombinant DNA.

The Notch sequences provided by the instant invention include those nucleotide sequences encoding substantially the same amino acid sequences as found in native Notch protein, and those encoded amino acid sequences with
10 functionally equivalent amino acids, all as described in Section 5.6 *infra* for Notch derivatives.

Similar methods to those described *supra* can be used to obtain a nucleic acid encoding Delta, Serrate, or adhesive portions thereof, or other toporythmic gene of interest. In a specific embodiment, the Delta nucleic acid
15 has at least a portion of the sequence shown in Figure 1 (SEQ ID NO:1). In another specific embodiment, the Serrate nucleic acid has at least a portion of the sequence shown in Figure 5 (SEQ ID NO:3). The nucleic acid sequences encoding toporythmic proteins can be isolated from porcine, bovine, feline, avian, equine, or canine, as well as primate sources and any other species in which
20 homologs of known toporythmic genes [including but not limited to the following genes (with the publication of sequences in parentheses): Delta (Vassin et al., 1987, EMBO J. 6, 3431-3440; Kopczynski et al., 1988, Genes Dev. 2, 1723-1735; note corrections to the Kopczynski et al. sequence found in Figure 1 hereof (SEQ ID NO:1 and SEQ ID NO:2)) and Serrate (Fleming et al., 1990, Genes &
25 Dev. 4, 2188-2201)] can be identified. Such sequences can be altered by substitutions, additions or deletions that provide for functionally equivalent molecules, as described *supra*.

5.8. RECOMBINANT PRODUCTION OF PROTEIN THERAPEUTICS

30 The nucleic acid coding for a protein Therapeutic of the invention can be inserted into an appropriate expression vector, *i.e.*, a vector which

contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native toporythmic gene and/or its flanking regions. A variety of host-vector systems may be utilized to express the protein-coding
5 sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and
10 specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In a specific embodiment, the adhesive portion of the Notch gene, *e.g.*, that encoding EGF-like repeats (ELR) 11 and 12, is expressed. In other specific embodiments, the human Notch gene is expressed, or a sequence encoding a functionally active
15 portion of human Notch.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro*
20 recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a Notch protein or peptide fragment may be regulated by a second nucleic acid sequence so that the Notch protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a Notch protein may be controlled
25 by any promoter/enhancer element known in the art. Promoters which may be used to control toporythmic gene expression include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290, 304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22, 787-797), the herpes thymidine kinase
30 promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78, 1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature

296, 39-42); prokaryotic expression vectors such as the β -lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75, 3727-3731), or the *tac* promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80, 21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 5 1980, 242, 74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303, 209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 1981, Nucl. Acids Res. 9, 2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310, 115-120); promoter 10 elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et 15 al., 1984, Cell 38, 639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50, 399-409; MacDonald, 1987, Hepatology 7, 425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315, 115-122). immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38, 647-658; Adames et al., 1985, 20 Nature 318, 533-538; Alexander et al., 1987, Mol. Cell. Biol. 7, 1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45, 485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1, 268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf 25 et al., 1985, Mol. Cell. Biol. 5, 1639-1648; Hammer et al., 1987, Science 235, 53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1, 161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315, 338-340; Kollias et al., 1986, Cell 46, 89-94; myelin basic protein gene control region 30 which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48, 703-712); myosin light chain-2 gene control region which is active in skeletal

muscle (Sani, 1985, Nature 314, 283-286). and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234, 1372-1378).

Expression vectors containing Notch gene inserts can be identified
5 by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted toporythmic gene. In the second approach, the
10 recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if the Notch gene is inserted within the marker gene
15 sequence of the vector, recombinants containing the Notch insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the Notch gene product in *in vitro* assay
20 systems, *e.g.*, aggregation (adhesive) ability (see Sections 6-7, *infra*).

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously
25 explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors: bacteriophage vectors (*e.g.*, lambda). and plasmid and cosmid DNA vectors, to name but a few.

30 In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences. or modifies and processes the gene product

in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered Notch protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of a heterologous mammalian topotypic protein. Furthermore, different vector/host expression systems may effect processing reactions such as proteolytic cleavages to different extents.

In other specific embodiments, the Notch protein, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

In other embodiments, a Notch cDNA sequence may be chromosomally integrated and expressed. Homologous recombination procedures known in the art may be used.

5.8.1. IDENTIFICATION AND PURIFICATION OF THE EXPRESSED GENE PRODUCT

Once a recombinant which expresses the Notch gene sequence is identified, the gene product may be analyzed. This can be achieved by assays

based on the physical or functional properties of the product, including radioactive labelling of the product followed by analysis by gel electrophoresis.

Once the Notch protein is identified, it may be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable assay, including, but not limited to, aggregation assays (see Sections 6-7).

5.9. DERIVATIVES AND ANALOGS OF NOTCH AND OTHER TOPORYTHMIC PROTEINS

The invention further provides, as Therapeutics, derivatives (including but not limited to fragments) and analogs of Notch proteins. Also provided as Therapeutics are other toporythmic proteins and derivatives and analogs thereof, or Notch ligands, in particular, which promote or, alternatively, inhibit the interactions of such other toporythmic proteins with Notch.

The production and use of derivatives and analogs related to Notch are within the scope of the present invention. In a specific embodiment, the derivative or analog is functionally active, *i.e.*, capable of exhibiting one or more functional activities associated with a full-length, wild-type Notch protein. As one example, such derivatives or analogs which have the desired antigenicity can be used, for example, in diagnostic immunoassays as described in Section 5.3. Molecules which retain, or alternatively inhibit, a desired Notch property, *e.g.*, binding to Delta or other toporythmic proteins, binding to a intracellular ligand, can be used therapeutically as inducers, or inhibitors, respectively, of such property and its physiological correlates. Derivatives or analogs of Notch can be tested for the desired activity by procedures known in the art, including but not limited to the assays described *infra*. In one specific embodiment, peptide libraries can be screened to select a peptide with the desired activity; such screening can be carried out by assaying, *e.g.*, for binding to Notch or a Notch binding partner such as Delta.

In particular, Notch derivatives can be made by altering Notch sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a Notch gene may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of Notch genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the Notch derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a Notch protein including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Derivatives or analogs of Notch include but are not limited to those peptides which are substantially homologous to Notch or fragments thereof, or whose encoding nucleic acid is capable of hybridizing to a Notch nucleic acid sequence.

The Notch derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned Notch gene sequence can be modified by any of numerous strategies

known in the art (Maniatis, T., 1989, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analog of Notch, care should be taken to ensure that the modified gene remains within the same translational reading frame as Notch, uninterrupted by translational stop signals, in the gene region where the desired Notch activity is encoded.

Additionally, the Notch-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem 253:6551), use of TAB® linkers (Pharmacia), etc.

Manipulations of the Notch sequence may also be made at the protein level. Included within the scope of the invention are Notch protein fragments or other derivatives or analogs which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In addition, analogs and derivatives of Notch can be chemically synthesized. For example, a peptide corresponding to a portion of a Notch protein which comprises the desired domain, or which mediates the desired aggregation activity *in vitro*, or binding to a receptor, can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into

the Notch sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α -amino isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, designer amino acids
5 such as β -methyl amino acids, C α -methyl amino acids, and N α -methyl amino acids.

In a specific embodiment, the Notch derivative is a chimeric, or fusion, protein comprising a Notch protein or fragment thereof fused via a peptide bond at its amino- and/or carboxy-terminus to a non-Notch amino acid sequence.

10 In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a Notch-coding sequence joined in-frame to a non-Notch coding sequence). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in
15 the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. In a specific embodiment, a chimeric nucleic acid encoding a mature Notch protein with a heterologous signal sequence is expressed such that the chimeric protein is
20 expressed and processed by the cell to the mature Notch protein. As another example, and not by way of limitation, a recombinant molecule can be constructed according to the invention, comprising coding portions of both Notch and another toporythmic gene, *e.g.*, Delta. The encoded protein of such a recombinant molecule could exhibit properties associated with both Notch and
25 Delta and portray a novel profile of biological activities, including agonists as well as antagonists. The primary sequence of Notch and Delta may also be used to predict tertiary structure of the molecules using computer simulation (Hopp and Woods, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828); Notch/Delta chimeric recombinant genes could be designed in light of correlations between
30 tertiary structure and biological function. Likewise, chimeric genes comprising portions of Notch fused to any heterologous (non-Notch) protein-encoding

sequences may be constructed. A specific embodiment relates to a chimeric protein comprising a fragment of Notch of at least six amino acids.

In another specific embodiment, the Notch derivative is a fragment of Notch comprising a region of homology with another toporythmic protein. As
5 used herein, a region of a first protein shall be considered "homologous" to a second protein when the amino acid sequence of the region is at least 30% identical or at least 75% either identical or involving conservative changes, when compared to any sequence in the second protein of an equal number of amino acids as the number contained in the region.

10 Derivatives of Serrate, Delta, other toporythmic proteins, and the adhesive portions thereof, can be made by methods similar to those described *supra*.

15 5.9.1. DERIVATIVES OF NOTCH CONTAINING ONE OR MORE DOMAINS OF THE PROTEIN

In a specific embodiment, the invention provides Therapeutics that are Notch derivatives and analogs, in particular Notch fragments and derivatives of such fragments, that comprise one or more domains of the Notch protein, including but not limited to the extracellular domain, transmembrane domain,
20 intracellular domain, membrane-associated region, one or more of the EGF-like repeats (ELR) of the Notch protein, the cdc10 repeats, and the Notch/lin-12 repeats. In specific embodiments, the Notch derivative may lack all or a portion of the ELRs, or one or more other regions of the protein.

In a specific embodiment, relating to a Notch protein of a species
25 other than *D. melanogaster*, preferably human, fragments comprising specific portions of Notch are those comprising portions in the respective Notch protein most homologous to specific fragments of the *Drosophila* Notch protein (*e.g.*, ELR 11 and ELR 12).

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5.9.2. DERIVATIVES OF NOTCH OR OTHER
TOPORYTHMIC PROTEINS THAT MEDIATE
BINDING TO TOPORYTHMIC PROTEIN
DOMAINS, AND INHIBITORS THEREOF

5 The invention also provides Notch fragments, and analogs or derivatives of such fragments, which mediate binding to toporythmic proteins (and thus are termed herein "adhesive"), and nucleic acid sequences encoding the foregoing.

Also included as Therapeutics of the invention are toporythmic (e.g., Delta, Serrate) protein fragments, and analogs or derivatives thereof, which
10 mediate heterotypic binding to Notch (and thus are termed herein "adhesive"), and nucleic acid sequences relating to the foregoing.

Also included as Therapeutics of the invention are inhibitors (e.g., peptide inhibitors) of the foregoing toporythmic protein interactions with Notch.

The ability to bind to a toporythmic protein can be demonstrated
15 by *in vitro* aggregation assays with cells expressing such a toporythmic protein as well as cells expressing Notch or a Notch derivative (See Section 6). That is, the ability of a protein fragment to bind to a Notch protein can be demonstrated by detecting the ability of the fragment, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell. Inhibitors of
20 the foregoing interactions can be detected by their ability to inhibit such aggregation *in vitro*.

The nucleic acid sequences encoding toporythmic proteins or adhesive domains thereof, for use in such assays, can be isolated from human, porcine, bovine, feline, avian, equine, canine, or insect, as well as primate
25 sources and any other species in which homologs of known toporythmic genes can be identified.

In a specific embodiment, the adhesive fragment of Notch is that comprising the portion of Notch most homologous to ELR 11 and 12, i.e., amino acid numbers 447 through 527 (SEQ ID NO:14) of the *Drosophila* Notch
30 sequence (see Figure 4). In yet another specific embodiment, the adhesive fragment of Delta mediating binding to Notch is that comprising the portion of

Delta most homologous to about amino acid numbers 1-230 of the *Drosophila* Delta sequence (SEQ ID NO:2). In a specific embodiment relating to an adhesive fragment of Serrate, such fragment is that comprising the portion of Serrate most homologous to about amino acid numbers 85-283 or 79-282 of the *Drosophila* Serrate sequence (see Figure 5 (SEQ ID NO:4)).

Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as the adhesive sequences may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of the Notch, Delta, or Serrate genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the adhesive protein fragments or derivatives thereof, of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of the adhesive domains including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change.

Adhesive fragments of toporythmic proteins and potential derivatives, analogs or peptides related to adhesive toporythmic protein sequences, can be tested for the desired binding activity *e.g.*, by the *in vitro* aggregation assays described in the examples herein. Adhesive derivatives or adhesive analogs of adhesive fragments of toporythmic proteins include but are not limited to those peptides which are substantially homologous to the adhesive fragments, or whose encoding nucleic acid is capable of hybridizing to the nucleic acid sequence encoding the adhesive fragments, and which peptides and peptide analogs have positive binding activity *e.g.*, as tested *in vitro* by an aggregation assay such as described in the examples sections *infra*. Such derivatives and analogs are envisioned as Therapeutics and are within the scope of the present invention.

The adhesive-protein related derivatives, analogs, and peptides of the invention can be produced by various methods known in the art. The

manipulations which result in their production can occur at the gene or protein level (see Section 5.6).

Additionally, the adhesive-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*; and manipulations of the adhesive sequence may also
5 be made at the protein level (see Section 5.6).

In addition, analogs and peptides related to adhesive fragments can be chemically synthesized.

10 5.10. ASSAYS OF NOTCH PROTEINS, DERIVATIVES AND ANALOGS

The *in vitro* activity of Notch proteins, derivatives and analogs, and other toporythmic proteins which bind to Notch, can be assayed by various methods.

For example, in one embodiment, where one is assaying for the
15 ability to bind or compete with wild-type Notch for binding to anti-Notch antibody, various immunoassays known in the art can be used, including but not limited to competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions,
20 immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is
25 detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labelled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

30 In another embodiment, where one is assaying for the ability to mediate binding to Notch, one can carry out an *in vitro* aggregation assay such as

described *infra* in Section 6 or 7 (see also Fehon et al., 1990, Cell 61:523-534; Rebay et al., 1991, Cell 67:687-699).

In another embodiment, where another ligand for Notch is identified, ligand binding can be assayed, *e.g.*, by binding assays well known in the art. In another embodiment, physiological correlates of ligand binding to cells
5 expressing a Notch receptor (signal transduction) can be assayed.

In another embodiment, in insect or other model systems, genetic studies can be done to study the phenotypic effect of a Notch mutant that is a derivative or analog of wild-type Notch.

10 Other methods will be known to the skilled artisan and are within the scope of the invention.

5.11. ANTIBODIES TO NOTCH PROTEINS, DERIVATIVES AND ANALOGS

15 According to one embodiment of the invention, antibodies and fragments containing the binding domain thereof, directed against Notch are Therapeutics. Accordingly, Notch proteins, fragments or analogs or derivatives thereof, in particular, human Notch proteins or fragments thereof, may be used as immunogens to generate anti-Notch protein antibodies. Such antibodies can be
20 polyclonal, monoclonal, chimeric, single chain, Fab fragments, or from an Fab expression library. In a specific embodiment, antibodies specific to EGF-like repeats 11 and 12 of Notch may be prepared. In other embodiments, antibodies reactive with the extracellular domain of Notch can be generated. One example of such antibodies may prevent aggregation in an *in vitro* assay. In another
25 embodiment, antibodies specific to human Notch are produced.

Various procedures known in the art may be used for the production of polyclonal antibodies to a Notch protein or peptide. In a particular embodiment, rabbit polyclonal antibodies to an epitope of the human Notch protein encoded by a sequence depicted in Figure 10 or 11, or a subsequence
30 thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with the native Notch protein, or a synthetic version, or fragment thereof, including but not limited to rabbits, mice, rats, etc.

Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

For preparation of monoclonal antibodies directed toward a Notch protein sequence, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256, 495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4, 72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Antibody fragments which contain the idiotype (binding domain) of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize the adhesive domain of a Notch protein, one may assay generated hybridomas for a product which binds to a protein fragment containing such domain. For selection of an antibody specific to human Notch, one can select on the basis of positive binding to human Notch and a lack of binding to *Drosophila* Notch.

In addition to therapeutic utility, the foregoing antibodies have utility in diagnostic immunoassays as described in Section 5.6 *supra*.

Similar procedures to those described *supra* can be used to make Therapeutics which are antibodies to domains of other proteins (particularly toporythmic proteins) that bind or otherwise interact with Notch (*e.g.*, adhesive fragments of Delta or Serrate).

5

6. DOMAINS OF NOTCH MEDIATE BINDING WITH DELTA

Intermolecular association between the products of the Notch and Delta genes was detected by studying the effects of their expression on aggregation in *Drosophila* Schneider's 2 (S2) cells (Fehon et al., 1990, Cell 61, 523-534). Direct evidence of intermolecular interactions between Notch and Delta is described herein, as well as an assay system that can be used in dissecting the components of this interaction. Normally nonadhesive *Drosophila* S2 cultured cells that express Notch bind specifically in a calcium-dependent manner to cells that express Delta. Furthermore, while cells that express Notch do not bind to one another, cells that express Delta do bind to one another, suggesting that Notch and Delta can compete for binding to Delta at the cell surface. Notch and Delta form detergent-soluble complexes both in cultured cells and embryonic cells, suggesting that Notch and Delta interact directly at the molecular level in vitro and in vivo. The analyses suggest that Notch and Delta proteins interact at the cell surface via their extracellular domains.

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6.1. EXPERIMENTAL PROCEDURES

25

6.1.1. EXPRESSION CONSTRUCTS

Expression constructs are described in Fehon et al., 1990, Cell 61:523-534. Briefly, Notch encoded by the *MgIIa* minigene a cDNA/genomic chimeric construct (Ramos et al., 1989, Genetics 123, 337-348) was expressed following insertion into pRmHa-3 (Bunch, et al., 1988, Nucl. Acids Res. 16, 1043-1061). In the resulting construct, designated pMtNMg, the metallothionein promoter in pRmHa-3 is fused to Notch sequences starting 20 nucleotides upstream of the translation start site.

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The extracellular Notch construct (ECN1), was derived from a Notch cosmid (Ramos et al., 1989, Genetics 123, 337-348), and has an internal deletion of the Notch coding sequences from amino acids 1790 to 2625 inclusive (Wharton et al., 1985, Cell 43, 567-581), and a predicted frameshift that
5 produces a novel 59 amino acid carboxyl terminus.

For the Delta expression construct, the D11 cDNA (Kopczynski et al., 1988, Genes Dev. 2, 1723-1735; Figure 1; SEQ ID NO:1), which includes the complete coding capacity for Delta, was inserted into the EcoRI site of pRmHa-3. This construct was called pMTD11.

10

6.1.2. ANTIBODY PREPARATION

Hybridoma cell line C17.9C6 was obtained from a mouse immunized with a fusion protein based on a 2.1 kb Sall-HindIII fragment that includes coding sequences for most of the intracellular domain of Notch (amino
15 acids 1791-2504; Wharton et al., 1985, Cell 43, 567-581). The fragment was subcloned into pUR289 (Ruther and Muller-Hill, 1983, EMBO J. 2, 1791-1794), and then transferred into the pATH 1 expression vector (Dieckmann and Tzagoloff, 1985, J. Biol. Chem. 260, 1513-1520) as a BglII-HindIII fragment. Soluble fusion protein was expressed, precipitated by 25% (NH₄)₂SO₄,
20 resuspended in 6 M urea, and purified by preparative isoelectric focusing using a Rotofor (Bio-Rad) (for details, see Fehon, 1989, Rotofor Review No. 7, Bulletin 1518, Richmond, California: Bio-Rad Laboratories).

Mouse polyclonal antisera were raised against the extracellular domain of Notch using four BstYI fragments of 0.8 kb (amino acids 237-501:
25 Wharton et al., 1985, Cell 43, 567-581), 1.1 kb (amino acids 501-868), 0.99 kb (amino acids 868-1200), and 1.4 kb (amino acids 1465-1935) length, which spanned from the fifth EGF-like repeat across the transmembrane domain, singly inserted in-frame into the appropriate pGEX expression vector (Smith and Johnson, 1988, Gene 67, 31-40). Fusion proteins were purified on glutathione-
30 agarose beads (SIGMA). Mouse and rat antisera were precipitated with 50%

(NH₄)₂SO₄ and resuspended in PBS (150 mM NaCl, 14 mM Na₂HPO₄, 6 mM NaH₂PO₄) with 0.02% NaN₃.

Hybridoma cell line 201 was obtained from a mouse immunized with a fusion protein that includes coding sequences from the extracellular domain of Delta (Kopczynski et al., 1988, Genes Dev. 2, 1723-1735), including
5 sequences extending from the fourth through the ninth EGF-like repeats in Delta (amino acids 350-529).

Rat polyclonal antisera were obtained following immunization with antigen derived from the same fusion protein construct. In this case, fusion
10 protein was prepared by lysis of IPTG-induced cells in SDS-Laemmli buffer (Carroll and Laughon, 1987, in DNA Cloning, Volume III, D.M. Glover, ed. (Oxford: IRL Press), pp. 89-111), separation of proteins by SDS-PAGE, excision of the appropriate band from the gel, and electroelution of antigen from the gel slice for use in immunization (Harlow and Lane, 1988, Antibodies: A Laboratory
15 Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory)).

6.1.3. CELL CULTURE AND TRANSFECTION

The S2 cell line (Schneider, 1972, J. Embryol. Exp. Morph. 27, 353-365) was grown in M3 medium (prepared by Hazleton Co.) supplemented
20 with 2.5 mg/ml Bacto-Peptone (Difco), 1 mg/ml TC Yeastolate (Difco), 11% heat-inactivated fetal calf serum (FCS) (Hyclone), and 100 U/ml penicillin-100 µg/ml streptomycin-0.25 µg/ml fungizone (Hazleton). Cells growing in log phase at ~2 x 10⁶ cells/ml were transfected with 20 µg of DNA-calcium phosphate coprecipitate in 1 ml per 5 ml of culture as previously described (Wigler et al.,
25 1979, Proc. Natl. Acad. Sci. USA 78, 1373-1376), with the exception that BES buffer (SIGMA) was used in place of HEPES buffer (Chen and Okayama, 1987, Mol. Cell. Biol. 7, 2745-2752). After 16-18 hr, cells were transferred to conical centrifuge tubes, pelleted in a clinical centrifuge at full speed for 30 seconds, rinsed once with 1/4 volume of fresh complete medium, resuspended in their
30 original volume of complete medium, and returned to the original flask. Transfected cells were then allowed to recover for 24 hr before induction.

6.1.4. AGGREGATION ASSAYS

Expression of the Notch and Delta metallothionein constructs was induced by the addition of CuSO_4 to 0.7 mM. Cells transfected with the ECN1 construct were treated similarly. Cells were then mixed, incubated under
5 aggregation conditions, and scored for their ability to aggregate using specific antisera and immunofluorescence microscopy to visualize expressing cells.

Two types of aggregation assays were used. In the first assay, a total of 3 ml of cells ($5-10 \times 10^6$ cells/ml) was placed in a 25 ml Erlenmeyer flask and rotated at 40-50 rpm on a rotary shaker for 24-48 hr at room temperature.

10 For these experiments, cells were mixed 1-4 hr after induction began and induction was continued throughout the aggregation period. In the second assay, ~0.6 ml of cells were placed in a 0.6 ml Eppendorf tube (leaving a small bubble) after an overnight induction (12-16 hr) at room temperature and rocked gently for 1-2 hr at 4°C. The antibody inhibition and Ca^{2+} dependence experiments were
15 performed using the latter assay. For Ca^{2+} dependence experiments, cells were first collected and rinsed in balanced saline solution (BSS) with 11% FCS (BSS-FCS; FCS was dialyzed against 0.9% NaCl, 5mM Tris [pH 7.5]) or in Ca^{2+} free BSS-FCS containing 10 mM EGTA (Snow et al., 1989, Cell 59, 313-323) and then resuspended in the same medium at the original volume. For the antibody
20 inhibition experiments, Notch-transfected cells were collected and rinsed in M3 medium and then treated before aggregation in M3 medium for 1 hr at 4°C with a 1:250 dilution of immune or preimmune sera from each of the four mice immunized with fusion proteins containing segments from the extracellular domain of Notch (see Antibody Preparation above).

25

6.1.5. IMMUNOFLUORESCENCE

Cells were collected by centrifugation (3000 rpm for 20 seconds in an Eppendorf microcentrifuge) and fixed in 0.6 ml Eppendorf tubes with 0.5 ml of freshly made 2% paraformaldehyde in PBS for 10 min at room temperature.

30 After fixing, cells were collected by centrifugation, rinsed twice in PBS, and stained for 1 hr in primary antibody in PBS with 0.1% saponin (SIGMA) and 1%

normal goat serum (Pocono Rabbit Farm, Canadensis, PA). Monoclonal antibody supernatants were diluted 1:10 and mouse or rat sera were diluted 1:1000 for this step. Cells were then rinsed once in PBS and stained for 1 hr in specific secondary antibodies (double-labeling grade goat anti-mouse and goat anti-rat, Jackson Immunoresearch) in PBS-saponin-normal goat serum. After this incubation, cells were rinsed twice in PBS and mounted on slides in 90% glycerol, 10% 1 M Tris (pH 8.0), and 0.5% n-propyl gallate. Cells were viewed under epifluorescence on a Leitz Orthoplan 2 microscope.

Confocal micrographs were taken using the Bio-Rad MRC 500 system connected to a Zeiss Axiovert compound microscope. Images were collected using the BHS and GHS filter sets, aligned using the ALIGN program, and merged using MERGE. Fluorescent bleed-through from the green into the red channel was reduced using the BLEED program (all software provided by Bio-Rad). Photographs were obtained directly from the computer monitor using Kodak Ektar 125 film.

6.1.6. CELL LYSATES, IMMUNOPRECIPITATIONS, AND WESTERN BLOTS

Nondenaturing detergent lysates of tissue culture and wild-type Canton-S embryos were prepared on ice in ~10 cell vol of lysis buffer (300 mM NaCl, 50 mM Tris [pH 8.0], 0.5% NP-40, 0.5% deoxycholate, 1 mM CaCl_2 , 1 mM MgCl_2) with 1 mM phenylmethanesulfonyl fluoride (PMSF) and diisopropyl fluorophosphate diluted 1:2500 as protease inhibitors. Lysates were sequentially triturated using 18G, 21G, and 25G needles attached to 1 cc tuberculin syringes and then centrifuged at full speed in a microfuge 10 min at 4°C to remove insoluble material. Immunoprecipitation was performed by adding ~1 µg of antibody (1-2 µl of polyclonal antiserum) to 250-500 µl of cell lysate and incubating for 1 hr at 4°C with agitation. To this mixture, 15 µg of goat anti-mouse antibodies (Jackson Immunoresearch; these antibodies recognize both mouse and rat IgG) were added and allowed to incubate for 1 hr at 4°C with agitation. This was followed by the addition of 100 µl of fixed *Staphylococcus aureus* (Staph A) bacteria (Zysorbin, Zymed; resuspended according to

manufacturer's instructions), which had been collected, washed five times in lysis buffer, and incubated for another hour. Staph A-antibody complexes were then pelleted by centrifugation and washed three times in lysis buffer followed by two 15 min washes in lysis buffer. After being transferred to a new tube, precipitated material was suspended in 50 μ l of SDS-PAGE sample buffer, boiled immediately for 10 min, run on 3%-15% gradient gels, blotted to nitrocellulose, and detected using monoclonal antibodies and HRP-conjugated goat anti-mouse secondary antibodies as previously described (Johansen et al., 1989, J. Cell Biol. 109, 2427-2440). For total cellular protein samples used on Western blots, cells were collected by centrifugation, lysed in 10 cell vol of sample buffer that contained 1 mM PMSF, and boiled immediately.

6.2. RESULTS

6.2.1. THE EXPRESSION OF NOTCH AND DELTA IN CULTURED CELLS

To detect interactions between Notch and Delta, we examined the behavior of cells expressing these proteins on their surfaces using an aggregation assay. We chose the S2 cell line (Schneider, 1972, J. Embryol. Exp. Morph. 27, 353-365) for these studies. S2 cells express an aberrant Notch message and no detectable Notch due to a rearrangement of the 5' end of the Notch coding sequence. These cells also express no detectable Delta.

Results of Western blot and immunofluorescent analysis clearly showed that the Notch and Delta constructs support expression of proteins of the expected sizes and subcellular localization.

6.2.2. CELLS THAT EXPRESS NOTCH AND DELTA AGGREGATE

A simple aggregation assay was used to detect interactions between Notch and Delta expressed on the surface of S2 cells.

S2 cells in log phase growth were separately transfected with either the Notch or Delta metallothionein promoter construct. After induction with CuSO_4 , transfected cells were mixed in equal numbers and allowed to aggregate overnight at room temperature (for details, see Experimental

Procedures, Section 6.1). Alternatively, in some experiments intended to reduce metabolic activity, cells were mixed gently at 4°C for 1-2 hr. To determine whether aggregates had formed, cells were processed for immunofluorescence microscopy using antibodies specific for each gene product and differently labeled
5 fluorescent secondary antibodies. Expressing cells usually constituted less than 5% of the total cell population because transient rather than stable transformants were used. The remaining cells either did not express a given protein or expressed at levels too low for detection by immunofluorescence microscopy. As controls, we performed aggregations with only a single type of transfected cell.

10 The results (Fehon et al., 1990, Cell 61:523-534) showed that while Notch-expressing (Notch⁺) cells alone did not form aggregates in the assay, Delta-expressing (Delta⁺) cells did. The tendency for Delta⁺ cells to aggregate was apparent even in nonaggregated control samples, where cell clusters of 4-8 cells that probably arose from adherence between mitotic sister cells commonly
15 occurred. However, clusters were more common after incubation under aggregation conditions (*e.g.*, 19% of Delta⁺ cells in aggregates before incubation vs. 37% of Delta⁺ cells in aggregates after incubation), indicating that Delta⁺ cells are able to form stable contacts with one another in this assay.

In remarkable contrast to control experiments with Notch⁺ cells
20 alone, aggregation of mixtures of Notch⁺ and Delta⁺ cells resulted in the formation of clusters of up to 20 or more cells. The fraction of expressing cells found in clusters of four or more stained cells after 24 hr of aggregation ranged from 32%-54% in mixtures of Notch⁺ and Delta⁺ cells. This range was similar to that seen for Delta⁺ cells alone (37%-40%) but very different from that for
25 Notch⁺ cells alone (only 0%-5%). Although a few clusters that consisted only of Delta⁺ cells were found, Notch⁺ cells were never found in clusters of greater than four to five cells unless Delta⁺ cells were also present. Again, all cells within these clusters expressed either Notch or Delta, even though transfected cells composed only a small fraction of the total cell population. At 48 hr, the degree
30 of aggregation appeared higher (63%-71%), suggesting that aggregation had not yet reached a maximum after 24 hr under these conditions. Also, cells

cotransfected with Notch and Delta constructs (so that all transfected cells express both proteins) aggregated in a similar fashion under the same experimental conditions.

Notch involvement in the aggregation process was directly tested
5 by examining the effect of a mixture of polyclonal antisera directed against fusion proteins that spanned almost the entire extracellular domain of Notch on aggregation (see Experimental Procedures, Section 6.1). To minimize artifacts that might arise due to a metabolic response to patching of surface antigens, antibody treatment and the aggregation assay were performed at 4°C in these
10 experiments. Notch⁺ cells were incubated with either preimmune or immune mouse sera for 1 hr, Delta⁺ cells were added, and aggregation was performed for 1-2 hr. While Notch⁺ cells pretreated with preimmune sera aggregated with Delta⁺ cells (in one of three experiments, 23% of the Notch⁺ cells were in Notch⁺-Delta⁺ cell aggregates), those treated with immune sera did not (only 2%
15 of Notch⁺ cells were in aggregates). This result suggested that the extracellular domain of Notch was required for Notch⁺-Delta⁺ cell aggregation.

6.2.3. NOTCH-DELTA-MEDIATED AGGREGATION IS CALCIUM DEPENDENT

20 The ability of expressing cells to aggregate in the presence or absence of Ca²⁺ ions was tested to determine whether there is a Ca²⁺ ion requirement for Notch-Delta aggregation. To minimize possible nonspecific effects due to metabolic responses to the removal of Ca²⁺, these experiments were performed at 4°C. The results clearly demonstrated a dependence of Notch-
25 Delta-mediated aggregation on exogenous Ca²⁺.

6.2.4. NOTCH AND DELTA INTERACT WITHIN A SINGLE CELL

The question whether Notch and Delta are associated within the
30 membrane of one cell that expresses both proteins was posed by examining the distributions of Notch and Delta in cotransfected cells. To test whether the observed colocalization was coincidental or represented a stable interaction

between Notch and Delta, live cells were treated with an excess of polyclonal anti-Notch antiserum. This treatment resulted in "patching" of Notch on the surface of expressing cells into discrete patches as detected by immunofluorescence. There was a distinct correlation between the distributions of Notch and Delta on the surfaces of these cells after this treatment, indicating that these proteins are associated within the membrane.

6.2.5. INTERACTIONS WITH DELTA DO NOT REQUIRE THE INTRACELLULAR DOMAIN OF NOTCH

In addition to a large extracellular domain that contains EGF-like repeats, Notch has a sizeable intracellular (IC) domain of ~940 amino acids. The IC domain includes a phosphorylation site (Kidd et al., 1989, *Genes Dev.* 3, 1113-1129), a putative nucleotide binding domain, a polyglutamine stretch (Wharton et al., 1985, *Cell* 43, 567-581; Kidd, et al., 1986, *Mol. Cell. Biol.* 6, 3094-3108), and sequences homologous to the yeast *cdc10* gene, which is involved in cell cycle control in yeast (Breedon and Nasmyth, 1987, *Nature* 329, 651-654). A variant Notch construct was used from which coding sequences for ~835 amino acids of the IC domain, including all of the structural features noted above, had been deleted (leaving 25 membrane-proximal amino acids and a novel 59 amino acid carboxyl terminus; see Experimental Procedures).

In aggregation assays, cells that expressed the ECN1 construct consistently formed aggregates with Delta⁺ cells, but not with themselves, just as was observed for cells that expressed intact Notch. Sharp bands of ECN1 staining were observed within regions of contact with Delta⁺ cells, again indicating a localization of ECN1 within regions of contact between cells. To test for interactions within the membrane, surface antigen co-patching experiments were conducted using cells cotransfected with the ECN1 and Delta constructs. As observed for intact Notch, when ECN1 was patched using polyclonal antisera against the extracellular domain of Notch, ECN1 and Delta colocalized at the cell surface. These results demonstrate that the observed interactions between Notch and Delta within the membrane do not require the deleted portion of the IC domain of Notch and are therefore probably mediated by the extracellular domain.

6.2.6. NOTCH AND DELTA FORM DETERGENT-SOLUBLE INTERMOLECULAR COMPLEXES

The preceding results indicated molecular interactions between Notch and Delta present within the same membrane and between these proteins expressed on different cells. A further test for such interactions is whether these proteins would coprecipitate from nondenaturing detergent extracts of cells that express Notch and Delta. If Notch and Delta form a stable intermolecular complex either between or within cells, then it should be possible to precipitate both proteins from cell extracts using specific antisera directed against one of these proteins. This analysis was performed by immunoprecipitating Delta with polyclonal antisera from NP-40/deoxycholate lysates (see Experimental Procedures) of cells cotransfected with the Notch and Delta constructs that had been allowed to aggregate overnight or of 0-24 hr wild-type embryos.

Coprecipitation of Notch was detected in Delta immunoprecipitates from cotransfected cells and embryos. However, coprecipitating Notch appeared to be present in much smaller quantities than Delta and was therefore difficult to detect. The fact that immunoprecipitation of Delta results in the coprecipitation of Notch constitutes direct evidence that these two proteins form stable intermolecular complexes in transfected S2 cells and in embryonic cells.

6.3. DISCUSSION

Use of an in vitro aggregation assay that employs normally nonadhesive S2 cells showed that cells that express Notch and Delta adhere specifically to one another.

7. EGF REPEATS 11 AND 12 OF NOTCH ARE REQUIRED AND SUFFICIENT FOR NOTCH-DELTA-MEDIATED AGGREGATION

The same aggregation assay was used as described in Section 6, together with deletion mutants of Notch to identify regions within the extracellular domain of Notch necessary for interactions with Delta. The evidence shows that the EGF repeats of Notch are directly involved in this interaction and that only

two (ELR 11 and 12) of the 36 EGF repeats appear necessary. These two EGF repeats are sufficient for binding to Delta and that the calcium dependence of Notch-Delta mediated aggregation also associates with these two repeats. Finally, the two corresponding EGF repeats from the *Xenopus* homolog of Notch also mediate aggregation with Delta, implying that not only has the structure of Notch been evolutionarily conserved, but also its function. These results suggest that the extracellular domain of Notch is surprisingly modular, and could potentially bind a variety of proteins in addition to Delta. (See Rebay et al., 1991, Cell 67:687-699.)

7.1. EXPERIMENTAL PROCEDURES

7.1.1. EXPRESSION CONSTRUCTS

The constructs described are all derivatives of the full length Notch expression construct #1 pMtNMg (see Section 6, *supra*), and were made as described (Rebay et al., 1991, Cell 67:687-699).

7.1.2. CELL CULTURE AND TRANSFECTION

The *Drosophila* S2 cell line was grown and transfected as described in Section 6, *supra*. The Delta-expressing stably transformed S2 cell line L-49-6-7 (kindly established by L. Cherbas) was grown in M3 medium (prepared by Hazleton Co.) supplemented with 11% heat inactivated fetal calf serum (FCS) (Hyclone), 100 U/ml penicillin-100 µg/ml streptomycin-0.25 µg/ml fungizone (Hazleton), 2×10^{-7} M methotrexate, 0.1 mM hypoxanthine, and 0.016 mM thymidine.

7.1.3. AGGREGATION ASSAYS AND IMMUNOFLUORESCENCE

Aggregation assays and Ca^{++} dependence experiments were as described *supra*, Section 6. Cells were stained with the anti-Notch monoclonal antibody 9C6.C17 and anti-Delta rat polyclonal antisera (details described in Section 6, *supra*). Surface expression of Notch constructs in unpermeabilized cells was assayed using rat polyclonal antisera raised against the 0.8 kb (amino acids

237-501; Wharton et al., 1985, Cell 43, 567-581) BstYI fragment from the extracellular domain of Notch. Cells were viewed under epifluorescence on a Leitz Orthoplan 2 microscope.

5

7.2. RESULTS

7.2.1. EGF REPEATS 11 AND 12 OF NOTCH ARE REQUIRED FOR NOTCH-DELTA MEDIATED AGGREGATION

An extensive deletion analysis was undertaken of the extracellular domain of the Notch protein, which was shown (*supra*, Section 6; Fehon et al., 1990, Cell 61:523-534) to be involved in Notch-Delta interactions, to identify the precise domain of Notch mediating these interactions. The ability of cells transfected with the various deletion constructs to interact with Delta was tested using the aggregation assay described in Section 6. Briefly, Notch deletion constructs were transiently transfected into *Drosophila* S2 cells, induced with CuSO₄, and then aggregated overnight at room temperature with a small amount of cells from the stably transformed Delta expressing cell line L49-6-7(Cherbas), yielding a population typically composed of ~1% Notch expressing cells and ~5% Delta expressing cells, with the remaining cells expressing neither protein.

Schematic drawings of the constructs tested and results of the aggregation experiments are shown in Figure 2. To assay the degree of aggregation, cells were stained with antisera specific to each gene product and examined with immunofluorescent microscopy. Aggregates were defined as clusters of four or more cells containing both Notch and Delta expressing cells, and the values shown in Figure 2 represent the percentage of all Notch expressing cells found in such clusters. All numbers reflect the average result from at least two separate transfection experiments in which at least 100 Notch expressing cell units (either single cells or clusters) were scored.

The initial constructs (#2 DSph and #3 ΔCla) deleted large portions of the EGF repeats. Their inability to promote Notch-Delta aggregation suggested that the EGF repeats of Notch were involved in the interaction with Delta. A series of six in-frame ClaI restriction sites was used to further dissect

the region between EGF repeats 7 and 30. Due to sequence homology between repeats, five of the ClaI sites occur in the same relative place within the EGF repeat, just after the third cysteine, while the sixth site occurs just before the first cysteine of EGF repeat 31 (Figure 3). Thus, by performing a partial ClaI digestion and then religating, deletions were obtained that not only preserved the open reading frame of the Notch protein but in addition frequently maintained the structural integrity and conserved spacing, at least theoretically, of the three disulfide bonds in the chimeric EGF repeats produced by the religation (Figure 2, constructs #4-14). Unfortunately, the most 3' ClaI site was resistant to digestion while the next most 3' ClaI site broke between EGF repeats 30 and 31. Therefore, when various ClaI digestion fragments were reinserted into the framework of the complete ClaI digest (construct #3 ΔCla), the overall structure of the EGF repeats was apparently interrupted at the 3' junction.

Several points about this series of constructs are worth noting. First, removal of the ClaI restriction fragment breaking in EGF repeats 9 and 17 (construct #8 ΔEGF9-17) abolished aggregation with Delta, while reinsertion of this piece into construct #3 ΔCla, which lacks EGF repeats 7-30, restored aggregation to roughly wild type levels (construct #13 ΔCla+EGF9-17), suggesting that EGF repeats 9 through 17 contain sequences important for binding Delta. Second, all constructs in this series (#4-14) were consistent with the binding site mapping to EGF repeats 9 through 17. Expression constructs containing these repeats (#6, 7, 9, 10, 13) promoted Notch-Delta interactions while constructs lacking these repeats (#4, 5, 8, 11, 12, 14) did not. To confirm that inability to aggregate with Delta cells was not simply due to failure of the mutagenized Notch protein to reach the cell surface, but actually reflected the deletion of the necessary binding site, cell surface expression of all constructs was tested by immunofluorescently staining live transfected cells with antibodies specific to the extracellular domain of Notch. All constructs failing to mediate Notch-Delta interactions produced a protein that appeared to be expressed normally at the cell surface. Third, although the aggregation assay is not quantitative, two constructs which contained EGF repeats 9-17, #9 ΔEGF17-26 or

most noticeably #10 Δ EGF26-30, aggregated at a seemingly lower level. Cells transfected with constructs #9 Δ EGF17-26 and 10 Δ EGF26-30 showed considerably less surface staining than normal, although fixed and permeabilized cells reacted with the same antibody stained normally, indicating the epitopes
5 recognized by the antisera had not been simply deleted. By comparing the percentage of transfected cells in either permeabilized or live cell populations, it was found that roughly 50% of transfected cells for construct #9 Δ EGF17-26 and 10% for construct #10 Δ EGF26-30 produced detectable protein at the cell surface. Thus these two constructs produced proteins which often failed to reach
10 the cell surface, perhaps because of misfolding, thereby reducing, but not abolishing, the ability of transfected cells to aggregate with Delta-expressing cells.

Having mapped the binding site to EGF repeats 9 through 17, further experiments (Rebay et al., 1991, Cell 67:687-699) revealed that EGF repeat 14 of Notch was not involved in the interactions with Delta modelled by
15 the tissue culture assay.

To further map the Delta binding domain within EGF repeats 9-17, specific oligonucleotide primers and the PCR technique were used to generate several subfragments of this region. Three overlapping constructs, #16, 17 and 18 were produced, only one of which, #16 Δ Cla+EGF9-13, when transfected
20 into S2 cells, allowed aggregation with Delta cells. Construct #19 Δ Cla+EGF(10-13), which lacks EGF repeat 9, further defined EGF repeats 10-13 as the region necessary for Notch-Delta interactions.

Constructs #20-24 represented attempts to break this domain down even further using the same PCR strategy (see Figure 3). Constructs #20
25 Δ Cla+EGF(11-13), in which EGF repeat 12 is the only entire repeat added, and #21 Δ Cla+EGF(10-12), in which EGF repeat 11 is the only entire repeat added, failed to mediate aggregation, suggesting that the presence of either EGF repeat 11 or 12 alone was not sufficient for Notch-Delta interactions. However, since
30 the 3' ligation juncture of these constructs interrupted the overall structure of the EGF repeats, it was possible that a short "buffer" zone was needed to allow the crucial repeat to function normally. Thus for example in construct #19

ΔCla+EGF(10-13), EGF repeat 12 might not be directly involved in binding to Delta but instead might contribute the minimum amount of buffer sequence needed to protect the structure of EGF repeat 11, thereby allowing interactions with Delta. Constructs #22-24 addressed this issue. Constructs #22
5 ΔCla+EGF(10-11), which did not mediate aggregation, and #23 ΔCla+EGF(10-12), which did, again suggested that both repeats 11 and 12 are required while the flanking sequence from repeat 13 clearly is not. Finally, construct #24
ΔCla+EGF(11-12), although now potentially structurally disrupted at the 5' junction, convincingly demonstrated that the sequences from EGF repeat 10 are
10 not crucial. Thus based on entirely consistent data from 24 constructs, EGF repeats 11 and 12 of Notch together define the smallest functional unit obtainable from this analysis that contains the necessary sites for binding to Delta in transfected S2 cells.

15 7.2.2. EGF REPEATS 11 AND 12 OF NOTCH
ARE SUFFICIENT FOR NOTCH-DELTA
MEDIATED AGGREGATION

The large ClaI deletion into which PCR fragments were inserted (#3 ΔCla) retains roughly 1/3 of the original 36 EGF repeats as well as the three
20 Notch/lin-12 repeats. While these are clearly not sufficient to promote aggregation, it is possible that they form a necessary framework within which specific EGF repeats can interact with Delta. To test whether only a few EGF repeats were in fact sufficient to promote aggregation, two constructs were designed, #25 ΔEGF which deleted all 36 EGF repeats except for the first two-thirds of repeat 1, and #30 ΔECN which deleted the entire extracellular portion of
25 Notch except for the first third of EGF repeat 1 and ~35 amino acids just before the transmembrane domain. Fragments which had mediated Notch-Delta aggregation in the background of construct #3 ΔCla, when inserted into construct #25 ΔEGF, were again able to promote interactions with Delta (constructs #26-30). Analogous constructs (#31,32) in which the Notch/lin-12 repeats were also
30 absent, again successfully mediated Notch-Delta aggregation. Thus EGF repeats 11 and 12 appear to function as independent modular units which are sufficient to

mediate Notch-Delta interactions in S2 cells, even in the absence of most of the extracellular domain of Notch.

5 7.2.3. EGF REPEATS 11 AND 12 OF NOTCH
 MAINTAIN THE CALCIUM DEPENDENCE OF
 NOTCH-DELTA MEDIATED AGGREGATION

 The ability of cells expressing certain deletion constructs to
 aggregate with Delta expressing cells was examined in the presence or absence of
 Ca⁺⁺ ions. The calcium dependence of the interaction was preserved in even the
10 smallest construct, consistent with the notion that the minimal constructs
 containing EGF repeats 11 and 12 bind to Delta in a manner similar to that of full
 length Notch.

15 7.2.4. THE DELTA BINDING FUNCTION OF EGF
 REPEATS 11 AND 12 OF NOTCH IS
 CONSERVED IN THE XENOPUS
 HOMOLOG OF NOTCH

 PCR primers based on the *Xenopus* Notch sequence (Coffman et
 al., 1990, Science 249, 1438-1441) were used to obtain an ~350 bp fragment
 from a *Xenopus* Stage 17 cDNA library that includes EGF repeats 11 and 12
20 flanked by half of repeats 10 and 13 on either side. This fragment was cloned
 into construct #3 ΔCla, and three independent clones were tested for ability to
 interact with Delta in the cell culture aggregation assay. Two of the clones,
 #33a&bΔCla+XEGF(10-13), when transfected into S2 cells were able to mediate
 Notch-Delta interactions at a level roughly equivalent to the analogous *Drosophila*
25 Notch construct #19ΔCla+EGF(10-13), and again in a calcium dependent manner
 (Table III). However, the third clone #33cΔCla+XEGF(10-13) failed to mediate
 Notch-Delta interactions although the protein was expressed normally at the cell
 surface as judged by staining live unpermeabilized cells. Sequence comparison of
 the *Xenopus* PCR product in constructs #33a and 33c revealed a missense
30 mutation resulting in a leucine to proline change (amino acid #453, Coffman, et
 al., 1990, Science 249, 1438-1441) in EGF repeat 11 of construct #33c.
 Although this residue is not conserved between *Drosophila* and *Xenopus* Notch

(Figure 8), the introduction of a proline residue might easily disrupt the structure of the EGF repeat, and thus prevent it from interacting properly with Delta.

Comparison of the amino acid sequence of EGF repeats 11 and 12 of *Drosophila* and *Xenopus* Notch reveals a high degree of amino acid identity, including the calcium binding consensus sequence (Figure 4, SEQ ID NO:1 and NO:2). However the level of homology is not strikingly different from that shared between most of the other EGF repeats, which overall exhibit about 50% identity at the amino acid level. This one to one correspondence between the individual EGF repeats of *Drosophila* and *Xenopus* Notch, together with the functional conservation of ELR 11 and 12, suggests that the 36 EGF repeats of Notch comprise a tandem area of conserved functional units.

7.3. DISCUSSION

An extensive deletion analysis of the extracellular domain of Notch was used to show that the regions of Notch containing EGF-homologous repeats 11 and 12 are both necessary and sufficient for Notch-Delta-mediated aggregation, and that this Delta binding capability has been conserved in the same two EGF repeats of *Xenopus* Notch. The finding that the aggregation mapped to EGF repeats 11 and 12 of Notch demonstrates that the EGF repeats of Notch also function as specific protein binding domains. EGF repeats 11 and 12 alone (#32ΔECN+EGF(11-12)) were sufficient to maintain the Ca⁺⁺ dependence of Notch-Delta interactions.

The various deletion constructs suggest that ELR 11 and ELR 12 function as a modular unit, independent of the immediate context into which they are placed. Thus, neither the remaining 34 EGF repeats nor the three Notch/lin-12 repeats appear necessary to establish a structural framework required for EGF repeats 11 and 12 to function. Interestingly, almost the opposite effect was observed: although the aggregation assay does not measure the strength of the interaction, as the binding site was narrowed down to smaller and smaller fragments, an increase was observed in the ability of the transfected cells to aggregate with Delta expressing cells, suggesting that the normal flanking EGF

sequences actually impede association between the proteins. The remaining 34 EGF repeats may also form modular binding domains for other proteins interacting with Notch at various times during development.

The finding that EGF repeats 11 and 12 of Notch form a discrete
5 Delta binding unit represents the first concrete evidence supporting the idea that each EGF repeat or small subset of repeats may play a unique role during development, possibly through direct interactions with other proteins. The homologies seen between the adhesive domain of Delta and Serrate (Figure 5) suggest that the homologous portion of Serrate is "adhesive" in that it mediates
10 binding to other topotypic proteins (see Section 8, *infra*). In addition, the gene scabrous, which encodes a secreted protein with similarity to fibrinogen, may interact with Notch.

In addition to the EGF repeat, multiple copies of other structural motifs commonly occur in a variety of proteins. One relevant example is the
15 cdc10/ankyrin motif, six copies of which are found in the intracellular domain of Notch. Ankyrin contains 22 of these repeats. Perhaps repeated arrays of structural motifs may in general represent a linear assembly of a series of modular protein binding units. Given these results together with the known structural, genetic and developmental complexity of Notch, Notch may interact
20 with a number of different ligands in a precisely regulated temporal and spatial pattern throughout development. Such context specific interactions with extracellular proteins could be mediated by the EGF and Notch/lin-12 repeats, while interactions with cytoskeletal and cytoplasmic proteins could be mediated by the intracellular cdc10/ankyrin motifs.

25

8. SEQUENCES WHICH MEDIATE NOTCH-SERRATE INTERACTIONS

As described herein, the two EGF repeats of Notch which mediate
30 interactions with Delta, namely EGF repeats 11 and 12, also constitute a Serrate binding domain (see Rebay et al., 1991, Cell 67:687-699).

35

To test whether Notch and Serrate directly interact, S2 cells were transfected with a Serrate expression construct and mixed with Notch expressing cells in an aggregation assay. For the Serrate expression construct, a synthetic primer containing an artificial BamHI site immediately 5' to the initiator AUG at position 442 (all sequence numbers are according to Fleming et al., 1990, *Genes & Dev.* 4:2188-2201) and homologous through position 464, was used in conjunction with a second primer from position 681-698 to generate a DNA fragment of ~260 base pairs. This fragment was cut with BamHI and KpnI (position 571) and ligated into Bluescript KS+ (Stratagene). This construct, BTSer5'PCR, was checked by sequencing, then cut with KpnI. The Serrate KpnI fragment (571 - 2981) was inserted and the proper orientation selected, to generate BTSer5'PCR-Kpn. The 5' SacII fragment of BTSer5'PCR-Kpn (SacII sites in Bluescript polylinker and in Serrate (1199)) was isolated and used to replace the 5' SacII fragment of cDNA C1 (Fleming et al., 1990, *Genes & Dev.* 4:2188-2201), thus regenerating the full length Serrate cDNA minus the 5' untranslated regions. This insert was isolated by a Sall and partial BamHI digestion and shuttled into the BamHI and Sall sites of pRmHa-3 to generate the final expression construct, Ser-mtn.

Serrate expressing cells adhered to Notch expressing cells in a calcium dependent manner (Figure 2 and Rebay et al., 1991, *supra*). However, unlike Delta, under the experimental conditions tested, Serrate did not appear to interact homotypically. In addition, no interactions were detected between Serrate and Delta.

A subset of Notch deletion constructs were tested, and showed that EGF repeats 11 and 12, in addition to binding to Delta, also mediate interactions with Serrate (Figure 2; Constructs #1, 7-10, 13, 16, 17, 19, 28, and 32). In addition, the Serrate-binding function of these repeats also appears to have been conserved in the corresponding two EGF repeats of *Xenopus* Notch (#33ΔC1a+XEGF(10-13)). These results unambiguously show that Notch interacts with both Delta and Serrate, and that the same two EGF repeats of Notch mediate both interactions. The Serrate region which is essential for the

Notch/Serrate aggregation was also defined. Deleting nucleotides 676-1287 (i.e. amino acids 79-282) (See Figure 5; SEQ ID NO:3 and NO:4) eliminates the ability of the Serrate protein to aggregate with Notch.

5 Notch and Serrate appear to aggregate less efficiently than Notch and Delta, perhaps because the Notch-Serrate interaction is weaker. One trivial explanation for this reduced amount of aggregation could be that the Serrate construct simply did not express as much protein at the cell surface as the Delta construct, thereby diminishing the strength of the interaction. Alternatively, the difference in strength of interaction may indicate a fundamental functional
10 difference between Notch-Delta and Notch-Serrate interactions that may be significant *in vivo*.

9. THE CLONING, SEQUENCING, AND EXPRESSION OF HUMAN NOTCH

15 9.1. ISOLATION AND SEQUENCING OF HUMAN NOTCH

Clones for the human Notch sequence were originally obtained using the polymerase chain reaction (PCR) to amplify DNA from a 17-18 week human fetal brain cDNA library in the Lambda Zap II vector (Stratagene).

The 400-bp fragment obtained in this manner was then used as a
20 probe with which to screen the same library for human Notch clones. The original screen yielded three unique clones, hN3k, hN2K, and hN5k, all of which were shown by subsequent sequence analysis to fall in the 3' end of human Notch (Figure 6). A second screen using the 5' end of hN3k as probe was undertaken to search for clones encompassing the 5' end of human Notch. One unique clone,
25 hN4k, was obtained from this screen, and preliminary sequencing data indicate that it contains most of the 5' end of the gene (Figure 6). Together, clones hN4k, hN3k and hN5k encompass about 10 kb of the human Notch homolog(s), beginning early in the EGF-repeats and extending into the 3' untranslated region of the gene. All three clones are cDNA inserts in the EcoRI site of pBluescript
30 SK⁻ (Stratagene). The host E. coli strain is XL1-Blue (see Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor

Laboratory, Cold Spring Harbor, New York, p. A12). An alignment of the human Notch sequences with *Drosophila* Notch is shown in Figure 7.

The sequence of various portions of Notch contained in the cDNA clones was determined (by use of Sequenase®, U.S. Biochemical Corp.) and is shown for hN2k and hN4k in Figures 8 (SEQ ID NO:5-7) and 9 (SEQ ID NO:8, 9), respectively. Further sequence analysis of hN2k revealed that it encodes a human Notch sequence overlapping that contained in hN5k.

The complete nucleotide sequences of the human Notch cDNA contained in hN3k and hN5k was determined by the dideoxy chain termination method using the Sequenase® kit (U.S. Biochemical Corp.). Those nucleotide sequences encoding human Notch, in the appropriate reading frame, were readily identified since there are no introns and translation in only one out of the three possible reading frames yields a sequence which, upon comparison with the published *Drosophila* Notch deduced amino acid sequence, yields a sequence with a substantial degree of homology to the *Drosophila* Notch sequence. The DNA and deduced protein sequences of the human Notch cDNA in hN3k and hN5k are presented in Figures 10 (SEQ ID NO:10, 11) and 11 (SEQ ID NO:12, 13), respectively. Clone hN3k encodes a portion of a Notch polypeptide starting at approximately the third Notch/lin-12 repeat to several amino acids short of the carboxy-terminal amino acid. Clone hN5k encodes a portion of a Notch polypeptide starting approximately before the cdc10 region through the end of the polypeptide, and also contains a 3' untranslated region.

Comparing the DNA and protein sequences presented in Figure 10 (SEQ ID NO:10, 11) with those in Figure 11 (SEQ ID NO:12, 13) reveals significant differences between the sequences, suggesting that hN3k and hN5k represent part of two distinct Notch-homologous genes. The data thus suggest that the human genome harbors more than one Notch-homologous gene. This is unlike *Drosophila*, where Notch appears to be a single-copy gene.

Comparison of the DNA and amino acid sequences of the human Notch homologs contained in hN3k and hN5k with the corresponding *Drosophila* Notch sequences (as published in Wharton et al., 1985, Cell 43:567-581) and

with the corresponding *Xenopus Notch* sequences (as published in Coffman et al., 1990, Science 249:1438-1441 or available from Genbank® (accession number M33874)) also revealed differences.

5 The amino acid sequence shown in Figure 10 (hN3k) was compared with the predicted sequence of the TAN-1 polypeptide shown in Figure 2 of Ellisen et al., August 1991, Cell 66:649-661. Some differences were found between the deduced amino acid sequences; however, overall the hN3k Notch polypeptide sequence is 99% identical to the corresponding TAN-1 region (TAN-1 amino acids 1455 to 2506). Four differences were noted: in the region
10 between the third Notch/lin-12 repeat and the first cdc10 motif, there is an arginine (hN3k) instead of an X (TAN-1 amino acid 1763); (2) there is a proline (hN3k) instead of an X (TAN-1, amino acid 1787); (3) there is a conservative change of an aspartic acid residue (hN3k) instead of a glutamic acid residue (TAN-1, amino acid 2495); and (4) the carboxyl-terminal region differs
15 substantially between TAN-1 amino acids 2507 and 2535.

The amino acid sequence shown in Figure 11 (hN5k) was compared with the predicted sequence of the TAN-1 polypeptide shown in Figure 2 of Ellisen et al., August 1991, Cell 66:649-661. Differences were found between the deduced amino acid sequences. The deduced Notch polypeptide of
20 hN5k is 79% identical to the TAN-1 polypeptide (64% identical to *Drosophila Notch*) in the cdc10 region that encompasses both the cc10 motif (TAN-1 amino acids 1860 to 2217) and the well-conserved flanking regions (Fig. 12). The cdc10 region covers amino acids 1860 through 2217 of the TAN-1 sequence. In addition, the hN5k encoded polypeptide is 65% identical to the TAN-1
25 polypeptide (44% identical to *Drosophila Notch*) at the carboxy-terminal end of the molecule containing a PEST (proline, glutamic acid, serine, threonine)-rich region (TAN-1 amino acids 2482 to 2551) (Fig. 12B). The stretch of 215 amino acids lying between the aforementioned regions is not well conserved among any of the Notch-homologous clones represented by hN3k, hN5k, and TAN-1.
30 Neither the hN5k polypeptide nor *Drosophila Notch* shows significant levels of amino acid identity to the other proteins in this region (e.g., hN5k/TAN-1 =

24% identity; hN5k/*Drosophila* Notch = 11% identity; TAN-1/*Drosophila* Notch = 17% identity). In contrast, *Xenopus* Notch (Xotch) (SEQ ID NO:16), rat Notch (SEQ ID NO:17), and TAN-1 (SEQ ID NO:18) continue to share significant levels of sequence identity with one another (*e.g.*, TAN-1/rat Notch = 75% identity, TAN-1/*Xenopus* Notch = 45% identity, rat Notch/*Xenopus* Notch = 50% identity).

Examination of the sequence of the intracellular domains of the vertebrate Notch homologs shown in Figure 12B revealed an unexpected finding: all of these proteins, including hN5k, contain a putative CcN motif, associated with nuclear targeting function, in the conserved region following the last of the six cdc10 repeats (Fig. 12B). Although *Drosophila* Notch lacks such a defined motif, closer inspection of its sequence revealed the presence of a possible bipartite nuclear localization sequence (Robbins et al., 1991, Cell 64:615-623), as well as of possible CK II and cdc2 phosphorylation sites, all in relative proximity to one another, thus possibly defining an alternative type of CcN motif (Fig. 12B).

To isolate clones covering the 5' end of hN (the human Notch homolog contained in part in hN5k), clone hN2k was used as a probe to screen 260,000 plaques of human fetal brain phage library, commercially available from Stratagene, for crosshybridizing clones. Four clones were identified and isolated using standard procedures (Maniatis et al., 1982, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). Four clones were also isolated by hybridization to the Notch-homologous sequence of Adams et al., 1992, Nature 355:632-655, which was obtained from the ATCC.

To isolate clones covering the 5' end of TAN-1, the human fetal brain library that is commercially available from Stratagene was screened for clones which would extend the sequence to the 5' end. 880,000 plaques were screened and four clones were identified which crosshybridized with the hN3k sequences. Sequencing confirmed the relative position of these sequences within the Notch protein encoded by TAN-1.

The 5' sequence of our isolated TAN-1 homolog has been determined through nucleotide number 972 (nucleotide number 1 being the A in the ATG initiation codon), and compared to the sequence as published by Ellisen et al. (1991, Cell 66:649-661). At nucleotide 559, our TAN-1 homolog has a G, whereas Ellisen et al. disclose an A, which change results in a different encoded amino acid. Thus, within the first 324 amino acids, our TAN-1-encoded protein differs from that taught by Ellisen et al., since our protein has a Gly at position 187, whereas Ellisen et al. disclose an Arg at that position (as presented in Figure 13.)

The full-length amino acid sequences of both the hN (SEQ ID NO:19) and TAN-1-encoded (SEQ ID NO:20) proteins, as well as Xenopus and Drosophila Notch proteins, are shown in Figure 13. The full-length DNA coding sequence (except for that encoding the initiator Met) (contained in SEQ ID NO:21) and encoded amino acid sequence (except that the initiator Met is not shown) (contained in SEQ ID NO:19) of hN are shown in Figure 17.

9.2. EXPRESSION OF HUMAN NOTCH

Expression constructs were made using the human Notch cDNA clones discussed in Section 9.1 above. In the cases of hN3k and hN2k, the entire clone was excised from its vector as an EcoRI restriction fragment and subcloned into the EcoRI restriction site of each of the three pGEX vectors (Glutathione S-Transferase expression vectors; Smith and Johnson, 1988, Gene 7, 31-40). This allows for the expression of the Notch protein product from the subclone in the correct reading frame. In the case of hN5k, the clone contains two internal EcoRI restriction sites, producing 2.6, 1.5 and 0.6 kb fragments. Both the 2.6 and the 1.5 kb fragments have also been subcloned into each of the pGEX vectors.

The pGEX vector system was used to obtain expression of human Notch fusion (chimeric) proteins from the constructs described below. The cloned Notch DNA in each case was inserted, in phase, into the appropriate pGEX vector. Each construct was then electroporated into bacteria (E. coli), and

was expressed as a fusion protein containing the Notch protein sequences fused to the carboxyl terminus of glutathione S-transferase protein. Expression of the fusion proteins was confirmed by analysis of bacterial protein extracts by polyacrylamide gel electrophoresis, comparing protein extracts obtained from bacteria containing the pGEX plasmids with and without the inserted Notch DNA. The fusion proteins were soluble in aqueous solution, and were purified from bacterial lysates by affinity chromatography using glutathione-coated agarose (since the carboxyl terminus of glutathione S-transferase binds to glutathione). The expressed fusion proteins were bound by an antibody to *Drosophila* Notch, as assayed by Western blotting.

The constructs used to make human Notch-glutathione S-transferase fusion proteins were as follows:

hNFP#2 - PCR was used to obtain a fragment starting just before the cdc10 repeats at nucleotide 192 of the hN5k insert to just before the PEST-rich region at nucleotide 1694. The DNA was then digested with BamHI and SmaI and the resulting fragment was ligated into pGEX-3. After expression, the fusion protein was purified by binding to glutathione agarose. The purified polypeptide was quantitated on a 4-15% gradient polyacrylamide gel. The resulting fusion protein had an approximate molecular weight of 83 kD.

hN3FP#1 - The entire hN3k DNA insert (nucleotide 1 to 3235) was excised from the Bluescript (SK) vector by digesting with EcoRI. The DNA was ligated into pGEX-3.

hN3FP#2 - A 3' segment of hN3k DNA (nucleotide 1847 to 3235) plus some of the polylinker was cut out of the Bluescript (SK) vector by digesting with XmaI. The fragment was ligated into pGEX-1.

Following purification, these fusion proteins are used to make either polyclonal and/or monoclonal antibodies to human Notch.

10. NOTCH EXPRESSION IN NORMAL AND MALIGNANT CELLS

Various human patient tissue samples and cell lines, representing both normal and a wide variety of malignant cells are assayed to detect and/or quantitate expression of Notch. Patient tissue samples are obtained from the pathology department at the Yale University School of Medicine.

The following assays are used to measure Notch expression in patient tissue samples: (a) Northern hybridization; (b) Western blots; (c) *in situ* hybridization; and (d) immunocytochemistry. Assays are carried out using standard techniques. Northern hybridization and *in situ* hybridization are carried out (i) using a DNA probe specific to the Notch sequence of clone hN3k; and (ii) using a DNA probe specific to the Notch sequence of clone hN5k. Western blots and immunocytochemistry are carried out using an antibody to *Drosophila* Notch protein (which also recognizes human Notch proteins).

Northern hybridization and Western blots, as described above, are also used to analyze numerous human cell lines, representing various normal or cancerous tissues. The cell lines tested are listed in Table 2.

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Table 2

HUMAN CELL LINES

	<u>Tissue/Tumor</u>	<u>Cell line</u>
25	Bone marrow	IM-9 KG-1
	Brain	A-172 HS 683 U-87MG TE 671
30	Breast	BT-20 Hs 578Bs MDA-MB-330

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	Colon	Caco-2 SW 48 T84 WiDr
5	Embryo	FHs 173We
	Kidney	A-498 A-704 Caki-2
10	Leukemia	ARH-77 KG-1
	Liver	Hep G2 WRL 68
	Lung	Calu-1 HLF-a SK-Lu-1
15	Lymphoblasts	CCRF-CEM HuT 78
	Lymphoma	Hs 445 MS116 U-937
20	Melanoma	A-375 G-361 Hs 294T SK-MEL-1
	Myeloma	IM-9 RPMI 8226
25	Neuroblastoma	IMR-32 SK-N-SH SK-N-MC
	Ovary	Caov-3 Caov-4 PA-1
30	Plasma Cells	ARH-77

	Sarcoma	A-204 A673 HOS
5	Skin	Amdur II BUD-8
	Testis	Tera-1 Tera-2
	Thymus	Hs67
10	Uterus	AN3 Ca HEC-1-A

Malignancies of malignant cell tissue types which are thus shown to specifically express Notch can be treated as described in Section 5.1 *et seq.*

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10.1. EXPRESSION OF HUMAN NOTCH PROTEIN IS INCREASED IN VARIOUS MALIGNANCIES

As described below, we have found that human Notch protein expression is increased in at least three human cancers, namely cervical, breast, and colon cancer. Immunocytochemical staining of tissue samples from cervical,
20 breast, and colon cancers of human patients showed clearly that the malignant tissue expresses high levels of Notch, at increased levels relative to non-malignant tissue sections. This broad spectrum of different neoplasias in which there is elevated Notch expression suggests that many more cancerous conditions will be seen to upregulate Notch.

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Slides of human tumor samples (for breast, colon, and cervical tumors) were obtained from the tissue bank of the Pathology Department, Yale Medical School. The stainings were done using monoclonal antibodies raised against the P1 and P4 fusion proteins which were generated from sequences of hN and TAN-1, respectively.

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The P1 and P4 fusion proteins were obtained by insertion of the desired human Notch sequence into the appropriate pGEX expression vector

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(Smith and Johnson, 1988, Gene 7:31-40; AMRAD Corp., Melbourne, Australia) and were affinity-purified according to the instructions of the manufacturer (AMRAD Corp.). For production of the P1 fusion protein, pGEX-2 was cut with BamHI and ligated to a concatamer which consists of three copies of a 518 bp BamHI-BglIII fragment of hN. Rats were immunized with the expressed protein and monoclonal antibodies were produced by standard procedures. For production of the P4 fusion protein, pGEX-2 was cut with BamHI and ligated to a concatamer which consists of three copies of a 473 bp BamHI-BglIII fragment of TAN-1. Rats were immunized with the expressed protein, and monoclonal antibodies were produced by standard procedures.

In all tumors examined, the Notch proteins encoded by both human Notch homologs TAN-1 and hN were present at increased levels in the malignant part of the tissue compared to the normal part. Representative stainings are shown in the pictures provided (Figs. 14-16).

The staining procedure was as follows: The tissues were fixed in paraformaldehyde, embedded in paraffin, cut in 5 micrometer thick sections and placed on glass slides. Then the following steps were carried out:

1. Deparaffinization through 4 changes of xylene, 4 minutes each.
2. Removal of xylene through 3 changes in absolute ethanol, 4 minutes each.
3. Gradual rehydration of the tissues by immersing the slides into 95%, 90%, 80%, 60% and 30% ethanol, 4 minutes each. At the end the slides were rinsed in distilled water for 5 minutes.
4. Quenching of endogenous, peroxidase by incubating for 30 minutes in 0.3% hydrogen peroxide in methanol.
5. Washing in PBS (10 mM sodium phosphate pH 7.5, 0.9% NaCl) for 20 minutes.
6. Incubation for 1 hour in blocking solution. (Blocking solution: PBS containing 4% normal rabbit serum and 0.1 Triton X-100.)

11. DEPOSIT OF MICROORGANISMS

The following recombinant bacteria, each carrying a plasmid encoding a portion of human Notch, were deposited on May 2, 1991 with the American Type Culture Collection, 1201 Parklawn Drive, Rockville, Maryland 20852, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures.

	<u>Bacteria</u>	carrying	<u>Plasmid</u>	<u>ATCC Accession No.</u>
10	<u>E. coli</u> XL1-Blue		hN4k	68610
	<u>E. coli</u> XL1-Blue		hN3k	68609
	<u>E. coli</u> XL1-Blue		hN5k	68611

The present invention is not to be limited in scope by the microorganisms deposited or the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

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7. Incubation overnight at 4°C with primary antibody diluted in blocking solution. Final concentration of primary antibody 20-50 $\mu\text{g/ml}$.
8. Washing for 20 minutes with PBS+0.1% Triton X-100 (3 changes).
9. Incubation for 30 minutes with biotinylated rabbit anti-rat antibody: 50 μl of biotinylated antibody (VECTOR) in 10 ml of blocking solution.
10. Washing for 20 minutes with PBS+0.1% Triton X-100 (3 changes).
11. Incubation with ABC reagent (VECTOR) for 30 minutes (the reagent is made in PBS+0.1% Triton X-100).
12. Washing for 20 minutes in PBS+0.1% Triton X-100. Followed by incubation for 2 minutes in PBS+0.5% Triton X-100.
13. Incubation for 2-5 minutes in peroxidase substrate solution. Peroxidase substrate solution: Equal volumes of 0.02% hydrogen peroxide in distilled water and 0.1% diaminobenzidine tetrahydrochloride (DAB) in 0.1 M Tris buffer pH 7.5 are mixed just before the incubation with the tissues. Triton X-100 is added to the final solution at a concentration of 0.5%.
14. Washing for 15 minutes in tap water.
15. Counterstaining for 10 minutes with Mayer's hematoxylin.
16. Washing for 15 minutes in tap water.
17. Dehydration through changes in 30%, 60%, 80%, 90%, 95% and absolute ethanol (4 minutes each).
18. Immersion into xylene (2 changes, 4 minutes each).
19. Mounting, light microscopy.

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 88, lines 1-12 of the description *

A. IDENTIFICATION OF DEPOSIT *

Further deposits are identified on an additional sheet *

Name of depositary institution *

American Type Culture Collection

Address of depositary institution (including postal code and country) *

12301 Parklawn Drive
Rockville, MD 20852
USDate of deposit * May 2, 1991 Accession Number * 68610**B. ADDITIONAL INDICATIONS** * (leave blank if not applicable). This information is continued on a separate attached sheet**C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *** (of the indications are not all designated States)**D. SEPARATE FURNISHING OF INDICATIONS *** (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☒ This sheet was received with the International application when filed (to be checked by the receiving Office)
(Authorized Officer)☐ The date of receipt (from the applicant) by the International Bureau *

was

(Authorized Officer)

- 88.2 -

International Application No: PCT/ /

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive
Rockville, MD 20852
US

Accession No.

68609

68611

Date of Deposit

May 2, 1991

May 2, 1991

-89-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Artavanis-Tsakonas, S. et al.
- (ii) TITLE OF INVENTION: Therapeutic And Diagnostic Methods
And Compositions Based On Notch Proteins And
Nucleic Acids
- (iii) NUMBER OF SEQUENCES: 21
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Pennie & Edmonds
 - (B) STREET: 1155 Avenue of the Americas
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 10036
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: To be assigned
 - (B) FILING DATE: On even date
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Misrock, S. Leslie
 - (B) REGISTRATION NUMBER: 18,872
 - (C) REFERENCE/DOCKET NUMBER: 7326-018
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 212 790-9090
 - (B) TELEFAX: 212 8698864/9741
 - (C) TELEX: 66141 PENNIE

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2892 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 142..2640

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCGGAG GAATTATTCA AAACATAAAC ACAATAAACA ATTTGAGTAG TTGCCGCACA	60
CACACACACA CACAGCCCGT GGATTATTAC ACTAAAAGCG ACACTCAATC CAAAAAATCA	120
GCAACAAAAA CATCAATAAA C ATG CAT TGG ATT AAA TGT TTA TTA ACA GCA	171

Met His Trp Ile Lys Cys Leu Leu Thr Ala
1 5 10

ENSDOCID: -WO 9407474A1_1

-91-

ATC	CAT	GGC	ACC	TGC	AAC	AAA	CCC	TGG	ACT	TGC	ATC	TGC	AAC	GAG	GGT	987
Ile	His	Gly	Thr	Cys	Asn	Lys	Pro	Trp	Thr	Cys	Ile	Cys	Asn	Glu	Gly	
			270					275						280		
TGG	GGA	GGC	TTG	TAC	TGC	AAC	CAG	GAT	CTG	AAC	TAC	TGC	ACC	AAC	CAC	1035
Trp	Gly	Gly	Leu	Tyr	Cys	Asn	Gln	Asp	Leu	Asn	Tyr	Cys	Thr	Asn	His	
			285				290						295			
AGA	CCC	TGC	AAG	AAT	GGC	GGA	ACC	TGC	TTC	AAC	ACC	GGC	GAG	GGA	TTG	1083
Arg	Pro	Cys	Lys	Asn	Gly	Gly	Thr	Cys	Phe	Asn	Thr	Gly	Glu	Gly	Leu	
	300					305					310					
TAC	ACA	TGC	AAA	TGC	GCT	CCA	GGA	TAC	AGT	GGT	GAT	GAT	TGC	GAA	AAT	1131
Tyr	Thr	Cys	Lys	Cys	Ala	Pro	Gly	Tyr	Ser	Gly	Asp	Asp	Cys	Glu	Asn	
					320					325					330	
GAG	ATC	TAC	TCC	TGC	GAT	GCC	GAT	GTC	AAT	CCC	TGC	CAG	AAT	GGT	GGT	1179
Glu	Ile	Tyr	Ser	Cys	Asp	Ala	Asp	Val	Asn	Pro	Cys	Gln	Asn	Gly	Gly	
				335					340					345		
ACC	TGC	ATC	GAT	GAG	CCG	CAC	ACA	AAA	ACC	GGC	TAC	AAG	TGT	CAT	TGC	1227
Thr	Cys	Ile	Asp	Glu	Pro	His	Thr	Lys	Thr	Gly	Tyr	Lys	Cys	His	Cys	
			350					355					360			
GCC	AAC	GGC	TGG	AGC	GGA	AAG	ATG	TGC	GAG	GAG	AAA	GTG	CTC	ACG	TGT	1275
Ala	Asn	Gly	Trp	Ser	Gly	Lys	Met	Cys	Glu	Glu	Lys	Val	Leu	Thr	Cys	
			365				370					375				
TCG	GAC	AAA	CCC	TGT	CAT	CAG	GGA	ATC	TGC	CGC	AAC	GTT	CGT	CCT	GGC	1323
Ser	Asp	Lys	Pro	Cys	His	Gln	Gly	Ile	Cys	Arg	Asn	Val	Arg	Pro	Gly	
	380					385					390					
TTG	GGA	AGC	AAG	GGT	CAG	GGC	TAC	CAG	TGC	GAA	TGT	CCC	ATT	GGC	TAC	1371
Leu	Gly	Ser	Lys	Gly	Gln	Gly	Tyr	Gln	Cys	Glu	Cys	Pro	Ile	Gly	Tyr	
	395				400					405				410		
AGC	GGA	CCC	AAC	TGC	GAT	CTC	CAG	CTG	GAC	AAC	TGC	AGT	CCG	AAT	CCA	1419
Ser	Gly	Pro	Asn	Cys	Asp	Leu	Gln	Leu	Asp	Asn	Cys	Ser	Pro	Asn	Pro	
				415					420					425		
TGC	ATA	AAC	GGT	GGA	AGC	TGT	CAG	CCG	AGC	GGA	AAG	TGT	ATT	TGC	CCA	1467
Cys	Ile	Asn	Gly	Gly	Ser	Cys	Gln	Pro	Ser	Gly	Lys	Cys	Ile	Cys	Pro	
			430					435					440			
GCG	GGA	TTT	TCG	GGA	ACG	AGA	TGC	GAG	ACC	AAC	ATT	GAC	GAT	TGT	CTT	1515
Ala	Gly	Phe	Ser	Gly	Thr	Arg	Cys	Glu	Thr	Asn	Ile	Asp	Asp	Cys	Leu	
		445					450					455				
GGC	CAC	CAG	TGC	GAG	AAC	GGA	GGC	ACC	TGC	ATA	GAT	ATG	GTC	AAC	CAA	1563
Gly	His	Gln	Cys	Glu	Asn	Gly	Gly	Thr	Cys	Ile	Asp	Met	Val	Asn	Gln	
	460					465					470					
TAT	CGC	TGC	CAA	TGC	GTT	CCC	GGT	TTC	CAT	GGC	ACC	CAC	TGT	AGT	AGC	1611
Tyr	Arg	Cys	Gln	Cys	Val	Pro	Gly	Phe	His	Gly	Thr	His	Cys	Ser	Ser	
	475				480					485				490		
AAA	GTT	GAC	TTG	TGC	CTC	ATC	AGA	CCG	TGT	GCC	AAT	GGA	GGA	ACC	TGC	1659
Lys	Val	Asp	Leu	Cys	Leu	Ile	Arg	Pro	Cys	Ala	Asn	Gly	Gly	Thr	Cys	
				495				500					505			
TTG	AAT	CTC	AAC	AAC	GAT	TAC	CAG	TGC	ACC	TGT	CGT	GCG	GGA	TTT	ACT	1707
Leu	Asn	Leu	Asn	Asn	Asp	Tyr	Gln	Cys	Thr	Cys	Arg	Ala	Gly	Phe	Thr	
			510					515					520			
GGC	AAG	GAT	TGC	TCT	GTG	GAC	ATC	GAT	GAG	TGC	AGC	AGT	GGA	CCC	TGT	1755
Gly	Lys	Asp	Cys	Ser	Val	Asp	Ile	Asp	Glu	Cys	Ser	Ser	Gly	Pro	Cys	
		525					530					535				

-92-

CAT AAC GGC GGC ACT TGC ATG AAC CGC GTC AAT TCG TTC GAA TGC GTG His Asn Gly Gly Thr Cys Met Asn Arg Val Asn Ser Phe Glu Cys Val 540 545 550	1803
TGT GCC AAT GGT TTC AGG GGC AAG CAG TGC GAT GAG GAG TCC TAC GAT Cys Ala Asn Gly Phe Arg Gly Lys Gln Cys Asp Glu Glu Ser Tyr Asp 555 560 565 570	1851
TCG GTG ACC TTC GAT GCC CAC CAA TAT GGA GCG ACC ACA CAA GCG AGA Ser Val Thr Phe Asp Ala His Gln Tyr Gly Ala Thr Thr Gln Ala Arg 575 580 585	1899
GCC GAT GGT TTG ACC AAT GCC CAG GTA GTC CTA ATT GCT GTT TTC TCC Ala Asp Gly Leu Thr Asn Ala Gln Val Val Leu Ile Ala Val Phe Ser 590 595 600	1947
GTT GCG ATG CCT TTG GTG GCG GTT ATT GCG GCG TGC GTG GTC TTC TGC Val Ala Met Pro Leu Val Ala Val Ile Ala Ala Cys Val Val Phe Cys 605 610 615	1995
ATG AAG CGC AAG CGT AAG CGT GCT CAG GAA AAG GAC GAC GCG GAG GCC Met Lys Arg Lys Arg Lys Arg Ala Gln Glu Lys Asp Asp Ala Glu Ala 620 625 630	2043
AGG AAG CAG AAC GAA CAG AAT GCG GTG GCC ACA ATG CAT CAC AAT GGC Arg Lys Gln Asn Glu Gln Asn Ala Val Ala Thr Met His His Asn Gly 635 640 645 650	2091
AGT GGG GTG GGT GTA GCT TTG GCT TCA GCC TCT CTG GGC GGC AAA ACT Ser Gly Val Gly Val Ala Leu Ala Ser Ala Ser Leu Gly Gly Lys Thr 655 660 665	2139
GGC AGC AAC AGC GGT CTC ACC TTC GAT GGC GGC AAC CCG AAT ATC ATC Gly Ser Asn Ser Gly Leu Thr Phe Asp Gly Gly Asn Pro Asn Ile Ile 670 675 680	2187
AAA AAC ACC TGG GAC AAG TCG GTC AAC AAC ATT TGT GCC TCA GCA GCA Lys Asn Thr Trp Asp Lys Ser Val Asn Asn Ile Cys Ala Ser Ala Ala 685 690 695	2235
GCA GCG GCG GCG GCG GCA GCA GCG GCG GAC GAG TGT CTC ATG TAC GGC Ala Ala Ala Ala Ala Ala Ala Ala Ala Asp Glu Cys Leu Met Tyr Gly 700 705 710	2283
GGA TAT GTG GCC TCG GTG GCG GAT AAC AAC AAT GCC AAC TCA GAC TTT Gly Tyr Val Ala Ser Val Ala Asp Asn Asn Asn Ala Asn Ser Asp Phe 715 720 725 730	2331
TGT GTG GCT CCG CTA CAA AGA GCC AAG TCG CAA AAG CAA CTC AAC ACC Cys Val Ala Pro Leu Gln Arg Ala Lys Ser Gln Lys Gln Leu Asn Thr 735 740 745	2379
GAT CCC ACG CTC ATG CAC CGC GGT TCG CCG GCA GGC AGC TCA GCC AAG Asp Pro Thr Leu Met His Arg Gly Ser Pro Ala Gly Ser Ser Ala Lys 750 755 760	2427
GGA GCG TCT GGC GGA GGA CCG GGA GCG GCG GAG GGC AAG AGG ATC TCT Gly Ala Ser Gly Gly Gly Pro Gly Ala Ala Glu Gly Lys Arg Ile Ser 765 770 775	2475
GTT TTA GGC GAG GGT TCC TAC TGT AGC CAG CGT TGG CCC TCG TTG GCG Val Leu Gly Glu Gly Ser Tyr Cys Ser Gln Arg Trp Pro Ser Leu Ala 780 785 790	2523
GCG GCG GGA GTG GCC GGA GCC TGT TCA TCC CAG CTA ATG GCT GCA GCT Ala Ala Gly Val Ala Gly Ala Cys Ser Ser Gln Leu Met Ala Ala Ala 795 800 805 810	2571

-93-

TCG GCA GCG GGC AGC GGA GCG GGG ACG GCG CAA CAG CAG CGA TCC GTG 2619
 Ser Ala Ala Gly Ser Gly Ala Gly Thr Ala Gln Gln Gln Arg Ser Val
 815 820 825

GTC TGC GGC ACT CCG CAT ATG TAACTCCAAA AATCCGGAAG GGCTCCTGGT 2670
 Val Cys Gly Thr Pro His Met
 830

AAATCCGGAG AAATCCGCAT GGAGGAGCTG ACAGCACATA CACAAAGAAA AGACTGGGTT 2730
 GGGTTCAAAA TGTGAGAGAG ACGCCAAAAT GTTGTGTGTG ATTGAAGCAG TTTAGTCGTC 2790
 ACGAAAAATG AAAAATCTGT AACAGGCATA ACTCGTAAAC TCCCTAAAAA ATTTGTATAG 2850
 TAATTAGCAA AGCTGTGACC CAGCCGTTTC GATCCCGAAT TC 2892

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 833 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met His Trp Ile Lys Cys Leu Leu Thr Ala Phe Ile Cys Phe Thr Val
 1 5 10 15

Ile Val Gln Val His Ser Ser Gly Ser Phe Glu Leu Arg Leu Lys Tyr
 20 25 30

Phe Ser Asn Asp His Gly Arg Asp Asn Glu Gly Arg Cys Cys Ser Gly
 35 40 45

Glu Ser Asp Gly Ala Thr Gly Lys Cys Leu Gly Ser Cys Lys Thr Arg
 50 55 60

Phe Arg Val Cys Leu Lys His Tyr Gln Ala Thr Ile Asp Thr Thr Ser
 65 70 75 80

Gln Cys Thr Tyr Gly Asp Val Ile Thr Pro Ile Leu Gly Glu Asn Ser
 85 90 95

Val Asn Leu Thr Asp Ala Gln Arg Phe Gln Asn Lys Gly Phe Thr Asn
 100 105 110

Pro Ile Gln Phe Pro Phe Ser Phe Ser Trp Pro Gly Thr Phe Ser Leu
 115 120 125

Ile Val Glu Ala Trp His Asp Thr Asn Asn Ser Gly Asn Ala Arg Thr
 130 135 140

Asn Lys Leu Leu Ile Gln Arg Leu Leu Val Gln Gln Val Leu Glu Val
 145 150 155 160

Ser Ser Glu Trp Lys Thr Asn Lys Ser Glu Ser Gln Tyr Thr Ser Leu
 165 170 175

Glu Tyr Asp Phe Arg Val Thr Cys Asp Leu Asn Tyr Tyr Gly Ser Gly
 180 185 190

Cys Ala Lys Phe Cys Arg Pro Arg Asp Asp Ser Phe Gly His Ser Thr
 195 200 205

-94-

Cys Ser Glu Thr Gly Glu Ile Ile Cys Leu Thr Gly Trp Gln Gly Asp
 210 215 220
 Tyr Cys His Ile Pro Lys Cys Ala Lys Gly Cys Glu His Gly His Cys
 225 230 235 240
 Asp Lys Pro Asn Gln Cys Val Cys Gln Leu Gly Trp Lys Gly Ala Leu
 245 250 255
 Cys Asn Glu Cys Val Leu Glu Pro Asn Cys Ile His Gly Thr Cys Asn
 260 265 270
 Lys Pro Trp Thr Cys Ile Cys Asn Glu Gly Trp Gly Gly Leu Tyr Cys
 275 280 285
 Asn Gln Asp Leu Asn Tyr Cys Thr Asn His Arg Pro Cys Lys Asn Gly
 290 295 300
 Gly Thr Cys Phe Asn Thr Gly Glu Gly Leu Tyr Thr Cys Lys Cys Ala
 305 310 315 320
 Pro Gly Tyr Ser Gly Asp Asp Cys Glu Asn Glu Ile Tyr Ser Cys Asp
 325 330 335
 Ala Asp Val Asn Pro Cys Gln Asn Gly Gly Thr Cys Ile Asp Glu Pro
 340 345 350
 His Thr Lys Thr Gly Tyr Lys Cys His Cys Ala Asn Gly Trp Ser Gly
 355 360 365
 Lys Met Cys Glu Glu Lys Val Leu Thr Cys Ser Asp Lys Pro Cys His
 370 375 380
 Gln Gly Ile Cys Arg Asn Val Arg Pro Gly Leu Gly Ser Lys Gly Gln
 385 390 395 400
 Gly Tyr Gln Cys Glu Cys Pro Ile Gly Tyr Ser Gly Pro Asn Cys Asp
 405 410 415
 Leu Gln Leu Asp Asn Cys Ser Pro Asn Pro Cys Ile Asn Gly Gly Ser
 420 425 430
 Cys Gln Pro Ser Gly Lys Cys Ile Cys Pro Ala Gly Phe Ser Gly Thr
 435 440 445
 Arg Cys Glu Thr Asn Ile Asp Asp Cys Leu Gly His Gln Cys Glu Asn
 450 455 460
 Gly Gly Thr Cys Ile Asp Met Val Asn Gln Tyr Arg Cys Gln Cys Val
 465 470 475 480
 Pro Gly Phe His Gly Thr His Cys Ser Ser Lys Val Asp Leu Cys Leu
 485 490 495
 Ile Arg Pro Cys Ala Asn Gly Gly Thr Cys Leu Asn Leu Asn Asn Asp
 500 505 510
 Tyr Gln Cys Thr Cys Arg Ala Gly Phe Thr Gly Lys Asp Cys Ser Val
 515 520 525
 Asp Ile Asp Glu Cys Ser Ser Gly Pro Cys His Asn Gly Gly Thr Cys
 530 535 540
 Met Asn Arg Val Asn Ser Phe Glu Cys Val Cys Ala Asn Gly Phe Arg
 545 550 555 560
 Gly Lys Gln Cys Asp Glu Glu Ser Tyr Asp Ser Val Thr Phe Asp Ala

-95-

565										570					575				
His	Gln	Tyr	Gly	Ala	Thr	Thr	Gln	Ala	Arg	Ala	Asp	Gly	Leu	Thr	Asn				
			580					585					590						
Ala	Gln	Val	Val	Leu	Ile	Ala	Val	Phe	Ser	Val	Ala	Met	Pro	Leu	Val				
		595					600					605							
Ala	Val	Ile	Ala	Ala	Cys	Val	Val	Phe	Cys	Met	Lys	Arg	Lys	Arg	Lys				
		610				615					620								
Arg	Ala	Gln	Glu	Lys	Asp	Asp	Ala	Glu	Ala	Arg	Lys	Gln	Asn	Glu	Gln				
					630					635					640				
Asn	Ala	Val	Ala	Thr	Met	His	His	Asn	Gly	Ser	Gly	Val	Gly	Val	Ala				
				645					650					655					
Leu	Ala	Ser	Ala	Ser	Leu	Gly	Gly	Lys	Thr	Gly	Ser	Asn	Ser	Gly	Leu				
			660					665					670						
Thr	Phe	Asp	Gly	Gly	Asn	Pro	Asn	Ile	Ile	Lys	Asn	Thr	Trp	Asp	Lys				
		675					680					685							
Ser	Val	Asn	Asn	Ile	Cys	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala				
		690				695					700								
Ala	Ala	Ala	Asp	Glu	Cys	Leu	Met	Tyr	Gly	Gly	Tyr	Val	Ala	Ser	Val				
		705			710				715						720				
Ala	Asp	Asn	Asn	Asn	Ala	Asn	Ser	Asp	Phe	Cys	Val	Ala	Pro	Leu	Gln				
				725				730						735					
Arg	Ala	Lys	Ser	Gln	Lys	Gln	Leu	Asn	Thr	Asp	Pro	Thr	Leu	Met	His				
			740					745					750						
Arg	Gly	Ser	Pro	Ala	Gly	Ser	Ser	Ala	Lys	Gly	Ala	Ser	Gly	Gly	Gly				
		755					760					765							
Pro	Gly	Ala	Ala	Glu	Gly	Lys	Arg	Ile	Ser	Val	Leu	Gly	Glu	Gly	Ser				
		770				775					780								
Tyr	Cys	Ser	Gln	Arg	Trp	Pro	Ser	Leu	Ala	Ala	Ala	Gly	Val	Ala	Gly				
					790					795					800				
Ala	Cys	Ser	Ser	Gln	Leu	Met	Ala	Ala	Ala	Ser	Ala	Ala	Gly	Ser	Gly				
				805				810						815					
Ala	Gly	Thr	Ala	Gln	Gln	Gln	Arg	Ser	Val	Val	Cys	Gly	Thr	Pro	His				
			820				825						830						

Met

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1320 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
- (A) NAME/KEY: CDS
 - (B) LOCATION: 442..1320

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCGAGTCGAG CGCCGTGCTT CGAGCGGTGA TGAGCCCCTT TTCTGTCAAC GCTAAAGATC	60
TACAAAACAT CAGCGCCTAT CAAGTGGAAG TGTCAAGTGT GAACAAAACA AAAACGAGAG	120
AAGCACATAC TAAGGTCCAT ATAAATAATA AATAATAATT GTGTGTGATA ACAACATTAT	180
CCAAACAAAA CCAAACAAAA CGAAGGCAAA GTGGAGAAAA TGATACAGCA TCCAGAGTAC	240
GGCCGTTATT CAGCTATCCA GAGCAAGTGT AGTGTGGCAA AATAGAAACA AACAAAGGCA	300
CCAAAATCTG CATACTGGG CTAATTAAGG CTGCCCAGCG AATTTACATT TGTGTGGTGC	360
CAATCCAGAG TGAATCCGAA ACAAACCTCCA TCTAGATCGC CAACCAGCAT CACGCTCGCA	420
AACGCCCCCA GAATGTACAA A ATG TTT AGG AAA CAT TTT CGG CGA AAA CCA	471
Met Phe Arg Lys His Phe Arg Arg Lys Pro	1 5 10
GCT ACG TCG TCG TCG TTG GAG TCA ACA ATA GAA TCA GCA GAC AGC CTG	519
Ala Thr Ser Ser Ser Leu Glu Ser Thr Ile Glu Ser Ala Asp Ser Leu	15 20 25
GGA ATG TCC AAG AAG ACG GCG ACA AAA AGG CAG CGT CCG AGG CAT CGG	567
Gly Met Ser Lys Lys Thr Ala Thr Lys Arg Gln Arg Pro Arg His Arg	30 35 40
GTA CCC AAA ATC GCG ACC CTG CCA TCG ACG ATC CGC GAT TGT CGA TCA	615
Val Pro Lys Ile Ala Thr Leu Pro Ser Thr Ile Arg Asp Cys Arg Ser	45 50 55
TTA AAG TCT GCC TGC AAC TTA ATT GCT TTA ATT TTA ATA CTG TTA GTC	663
Leu Lys Ser Ala Cys Asn Leu Ile Ala Leu Ile Leu Ile Leu Leu Val	60 65 70
CAT AAG ATA TCC GCA GCT GGT AAC TTC GAG CTG GAA ATA TTA GAA ATC	711
His Lys Ile Ser Ala Ala Gly Asn Phe Glu Leu Glu Ile Leu Glu Ile	75 80 85 90
TCA AAT ACC AAC AGC CAT CTA CTC AAC GGC TAT TGC TGC GGC ATG CCA	759
Ser Asn Thr Asn Ser His Leu Leu Asn Gly Tyr Cys Cys Gly Met Pro	95 100 105
GCG GAA CTT AGG GCC ACC AAG ACG ATA GGC TGC TCG CCA TGC ACG ACG	807
Ala Glu Leu Arg Ala Thr Lys Thr Ile Gly Cys Ser Pro Cys Thr Thr	110 115 120
GCA TTC CGG CTG TGC CTG AAG GAG TAC CAG ACC ACG GAG CAG GGT GCC	855
Ala Phe Arg Leu Cys Leu Lys Glu Tyr Gln Thr Thr Glu Gln Gly Ala	125 130 135
AGC ATA TCC ACG GGC TGT TCG TTT GGC AAC GCC ACC ACC AAG ATA CTG	903
Ser Ile Ser Thr Gly Cys Ser Phe Gly Asn Ala Thr Thr Lys Ile Leu	140 145 150
GGT GGC TCC AGC TTT GTG CTC AGC GAT CCG GGT GTG GGA GCC ATT GTG	951
Gly Gly Ser Ser Phe Val Leu Ser Asp Pro Gly Val Gly Ala Ile Val	155 160 165 170
CTG CCC TTT ACG TTT CGT TGG ACG AAG TCG TTT ACG CTG ATA CTG CAG	999
Leu Pro Phe Thr Phe Arg Trp Thr Lys Ser Phe Thr Leu Ile Leu Gln	175 180 185
GCG TTG GAT ATG TAC AAC ACA TCC TAT CCA GAT GCG GAG AGG TTA ATT	1047

-97-

Ala	Leu	Asp	Met	Tyr	Asn	Thr	Ser	Tyr	Pro	Asp	Ala	Glu	Arg	Leu	Ile		
			190					195					200				
GAG	GAA	ACA	TCA	TAC	TCG	GGC	GTG	ATA	CTG	CCG	TCG	CCG	GAG	TGG	AAG		1095
Glu	Glu	Thr	Ser	Tyr	Ser	Gly	Val	Ile	Leu	Pro	Ser	Pro	Glu	Trp	Lys		
		205					210					215					
ACG	CTG	GAC	CAC	ATC	GGG	CGG	AAC	GCG	CGG	ATC	ACC	TAC	CGT	GTC	CGG		1143
Thr	Leu	Asp	His	Ile	Gly	Arg	Asn	Ala	Arg	Ile	Thr	Tyr	Arg	Val	Arg		
		220				225					230						
GTG	CAA	TGC	GCC	GTT	ACC	TAC	TAC	AAC	ACG	ACC	TGC	ACG	ACC	TTC	TGC		1191
Val	Gln	Cys	Ala	Val	Thr	Tyr	Tyr	Asn	Thr	Thr	Cys	Thr	Thr	Phe	Cys		
		235			240					245					250		
CGT	CCG	CGG	GAC	GAT	CAG	TTC	GGT	CAC	TAC	GCC	TGC	GGC	TCC	GAG	GGT		1239
Arg	Pro	Arg	Asp	Asp	Gln	Phe	Gly	His	Tyr	Ala	Cys	Gly	Ser	Glu	Gly		
			255						260					265			
CAG	AAC	CTC	TGC	CTG	AAT	GGC	TGG	CAG	GGC	GTC	AAC	TGC	GAG	GAG	GCC		1287
Gln	Lys	Leu	Cys	Leu	Asn	Gly	Trp	Gln	Gly	Val	Asn	Cys	Glu	Glu	Ala		
		270						275					280				
ATA	TGC	AAG	GCG	GGC	TGC	GAC	CCC	GTC	CAC	GGC							1320
Ile	Cys	Lys	Ala	Gly	Cys	Asp	Pro	Val	His	Gly							
		285					290										

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 293 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Phe	Arg	Lys	His	Phe	Arg	Arg	Lys	Pro	Ala	Thr	Ser	Ser	Ser	Leu		
1				5					10						15		
Glu	Ser	Thr	Ile	Glu	Ser	Ala	Asp	Ser	Leu	Gly	Met	Ser	Lys	Lys	Thr		
		20					25						30				
Ala	Thr	Lys	Arg	Gln	Arg	Pro	Arg	His	Arg	Val	Pro	Lys	Ile	Ala	Thr		
		35				40						45					
Leu	Pro	Ser	Thr	Ile	Arg	Asp	Cys	Arg	Ser	Leu	Lys	Ser	Ala	Cys	Asn		
		50			55					60							
Leu	Ile	Ala	Leu	Ile	Leu	Ile	Leu	Leu	Val	His	Lys	Ile	Ser	Ala	Ala		
	65			70					75						80		
Gly	Asn	Phe	Glu	Leu	Glu	Ile	Leu	Glu	Ile	Ser	Asn	Thr	Asn	Ser	His		
		85					90						95				
Leu	Leu	Asn	Gly	Tyr	Cys	Cys	Gly	Met	Pro	Ala	Glu	Leu	Arg	Ala	Thr		
		100					105						110				
Lys	Thr	Ile	Gly	Cys	Ser	Pro	Cys	Thr	Thr	Ala	Phe	Arg	Leu	Cys	Leu		
		115				120						125					
Lys	Glu	Tyr	Gln	Thr	Thr	Glu	Gln	Gly	Ala	Ser	Ile	Ser	Thr	Gly	Cys		
		130				135					140						
Ser	Phe	Gly	Asn	Ala	Thr	Thr	Lys	Ile	Leu	Gly	Gly	Ser	Ser	Phe	Val		

-98-

145		150		155		160
Leu Ser Asp Pro Gly Val Gly Ala Ile Val Leu Pro Phe Thr Phe Arg	165		170		175	
Trp Thr Lys Ser Phe Thr Leu Ile Leu Gln Ala Leu Asp Met Tyr Asn	180	185		190		
Thr Ser Tyr Pro Asp Ala Glu Arg Leu Ile Glu Glu Thr Ser Tyr Ser	195	200		205		
Gly Val Ile Leu Pro Ser Pro Glu Trp Lys Thr Leu Asp His Ile Gly	210	215		220		
Arg Asn Ala Arg Ile Thr Tyr Arg Val Arg Val Gln Cys Ala Val Thr	225	230		235		240
Tyr Tyr Asn Thr Thr Cys Thr Thr Phe Cys Arg Pro Arg Asp Asp Gln		245	250		255	
Phe Gly His Tyr Ala Cys Gly Ser Glu Gly Gln Lys Leu Cys Leu Asn	260		265		270	
Gly Trp Gln Gly Val Asn Cys Glu Glu Ala Ile Cys Lys Ala Gly Cys	275	280		285		
Asp Pro Val His Gly	290					

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 267 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGGTGGACTT CCTTCGTGTA TTGGTGGGAG CCCTCGGGAA CGGGGGGTAA CACTGAAAGG	60
TCGAGTACCC ATTTCCGTCA TAACGGGTTG GTCGCCCCCT AGGGGTCGGA GTCAGGTGGA	120
CGGGAGGTG ACAACGCCCG GGGGACGGGT GGTACATGGT GTAAGGTCTT TACCGGACCG	180
GGCAAACGGG TCACACCGAA AGGGGTGAAC GGTAAC TACG GGGTCGTCCT GCCCGTCCAT	240
CGAGTCTGGT AAGAGGGTCG CCTTAAG	267

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 574 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

-99-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAATTCCTTC CATTATACGT GACTTTTCTG AAACGTAGC CACCCTAGTG TCTCTAACTC	60
CCTCTGGAGT TTGTCAGCTT TGGTCTTTTC AAAGAGCAGG CTCTCTTCAA GCTCCTTAAT	120
GGGGGCATGC TCCAGTTTGG TCTGCGTCTC AAGATCACCT TTGGTAATTG ATTCTTCTTC	180
AACCCGGAAC TGAAGGCTGG CTCTCACCT CTAGGCAGAG CAGGAATTCC GAGGTGGATG	240
TGTTAGATGT GAATGTCCGT GGCCAGATG GCTGCACCCC ATTGATGTTG GCTTCTCTCC	300
GAGGAGGCAG CTCAGATTTG AGTGATGAAG ATGAAGATGC AGAGGACTGT TCTGCTAACA	360
TCATCACAGA CTTGGTCTAC CAGGGTGCCA GCCTCCAGNC CAGACAGACC GGACTGGTGA	420
GATGGCCCTG CACCTTGCAG CCCGCTACTC ACGGGCTGAT GCTGCCAAGC GTCTCCTGGA	480
TGCAGGTGCA GATGCCAATG CCCAGGACAA CATGGGCCGC TGTCCACTCC ATGCTGCAGT	540
GGCACGTGAT GCCAAGGTGT ATTCAGATCT GTTA	574

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 295 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCAGATTCT GATTGCAAC CGAGTAACTG ATCTAGATGC CAGGATGAAT GATGGTACTA	60
CACCCCTGAT CCTGGCTGCC CGCCTGGCTG TGGAGGGAAT GGTGGCAGAA CTGATCAACT	120
GCCAAGCGGA TGTGAATGCA GTGGATGACC ATGGAAAATC TGCTCTTCAC TGGGCAGCTG	180
CTGTCAATAA TGTGGAGGCA ACTCTTTTGT TGTGAAAAA TGGGGCCAAC CGAGACATGC	240
AGGACAACAA GGAAGAGACA CCTCTGTTTC TTGCTGCCCC GGAGGAGCTA TAAGC	295

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 248 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAATTCCATT CAGGAGGAAA GGGTGGGGAG AGAAGCAGGC ACCCACTTTC CCGTGGCTGG	60
ACTCGTTCCC AGGTGGCTCC ACCGGCAGCT GTGACCGCCG CAGGTGGGGG CGGAGTGCCA	120
TTCAGAAAAT TCCAGAAAAG CCCTACCCCA ACTCGGACGG CAACGTCACA CCCGTGGGTA	180

-100-

GCAACTGGCA CACAAACAGC CAGCGTGTCT GGGGCACGGG GGGATGGCAC CCCCTGCAGG 240
CAGAGCTG 248

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 323 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TACGTATCTC GAGCACAGAC AGCTGACGTA CACTTTTNNNA GTGCGAGGGA CATTCGTCCG 60
 ACCAGTACGA ACATTTAGGC TCAGTACGGT AGGTCCATGG CCAAGACTAG GAGACGTAGG 120
 GAGCTACAGG TCCCGCTCGC TAAACTCGGA CCACTGAAAC CTCCGGTCGA CAGTCGGTAA 180
 GCGAACAAGA GGGCCAGATC TTAGAGAAGG TGTCGCGGCG AGACTCGGGC TCGGGTCAGG 240
 CGGCCTTAAG GACGTCGGGC CCNNNAGGTG ATCAAGATCT CGNCNCGGCG GGCGCCACCT 300
 CGAGGNCGAA AACAAGGGAA ATC 323

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3234 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..3234

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGC CAG GAG GAC GCG GGC AAC AAG GTC TGC AGC CTG CAG TGC AAC AAC 48
 Cys Gln Glu Asp Ala Gly Asn Lys Val Cys Ser Leu Gln Cys Asn Asn
 1 5 10 15
 CAC GCG TGC GGC TGG GAC GGC GGT GAC TGC TCC CTC AAC TTC AAT GAC 96
 His Ala Cys Gly Trp Asp Gly Gly Asp Cys Ser Leu Asn Phe Asn Asp
 20 25 30
 CCC TGG AAG AAC TGC ACG CAG TCT CTG CAG TGC TGG AAG TAC TTC AGT 144
 Pro Trp Lys Asn Cys Thr Gln Ser Leu Gln Cys Trp Lys Tyr Phe Ser
 35 40 45
 GAC GGC CAC TGT GAC AGC CAG TGC AAC TCA GCC GGC TGC CTC TTC GAC 192
 Asp Gly His Cys Asp Ser Gln Cys Asn Ser Ala Gly Cys Leu Phe Asp
 50 55 60
 GGC TTT GAC TGC CAG CGT GCG GAA GGC CAG TGC AAC CCC CTG TAC GAC 240

-101-

Gly	Phe	Asp	Cys	Gln	Arg	Ala	Glu	Gly	Gln	Cys	Asn	Pro	Leu	Tyr	Asp	
65					70					75					80	
CAG	TAC	TGC	AAG	GAC	CAC	TTC	AGC	GAC	GGG	CAC	TGC	GAC	CAG	GGC	TGC	288
Gln	Tyr	Cys	Lys	Asp	His	Phe	Ser	Asp	Gly	His	Cys	Asp	Gln	Gly	Cys	
			85						90					95		
AAC	AGC	GCG	GAG	TGC	GAG	TGG	GAC	GGG	CTG	GAC	TGT	GCG	GAG	CAT	GTA	336
Asn	Ser	Ala	Glu	Cys	Glu	Trp	Asp	Gly	Leu	Asp	Cys	Ala	Glu	His	Val	
			100					105					110			
CCC	GAG	AGG	CTG	GCG	GCC	GGC	ACG	CTG	GTG	GTG	GTG	GTG	CTG	ATG	CCG	384
Pro	Glu	Arg	Leu	Ala	Ala	Gly	Thr	Leu	Val	Val	Val	Val	Leu	Met	Pro	
			115				120						125			
CCG	GAG	CAG	CTG	CGC	AAC	AGC	TCC	TTC	CAC	TTC	CTG	CGG	GAG	CTC	AGC	432
Pro	Glu	Gln	Leu	Arg	Asn	Ser	Phe	His	Phe	Leu	Arg	Glu	Leu	Ser		
			130			135					140					
CGC	GTG	CTG	CAC	ACC	AAC	GTG	GTC	TTC	AAG	CGT	GAC	GCA	CAC	GGC	CAG	480
Arg	Val	Leu	His	Thr	Asn	Val	Val	Phe	Lys	Arg	Asp	Ala	His	Gly	Gln	
145					150					155					160	
CAG	ATG	ATC	TTC	CCC	TAC	TAC	GGC	CGC	GAG	GAG	GAG	CTG	CGC	AAG	CAC	528
Gln	Met	Ile	Phe	Pro	Tyr	Tyr	Gly	Arg	Glu	Glu	Glu	Leu	Arg	Lys	His	
				165					170					175		
CCC	ATC	AAG	CGT	GCC	GCC	GAG	GGC	TGG	GCC	GCA	CCT	GAC	GCC	CTG	CTG	576
Pro	Ile	Lys	Arg	Ala	Ala	Glu	Gly	Trp	Ala	Ala	Pro	Asp	Ala	Leu	Leu	
			180					185					190			
GGC	CAG	GTG	AAG	GCC	TCG	CTG	CTC	CCT	GGT	GGC	AGC	GAG	GGT	GGG	CGG	624
Gly	Gln	Val	Lys	Ala	Ser	Leu	Leu	Pro	Gly	Gly	Ser	Glu	Gly	Gly	Arg	
		195				200						205				
CGG	CGG	AGG	GAG	CTG	GAC	CCC	ATG	GAC	GTC	CGC	GGC	TCC	ATC	GTC	TAC	672
Arg	Arg	Arg	Glu	Leu	Asp	Pro	Met	Asp	Val	Arg	Gly	Ser	Ile	Val	Tyr	
		210				215					220					
CTG	GAG	ATT	GAC	AAC	CGG	CAG	TGT	GTG	CAG	GCC	TCC	TCG	CAG	TGC	TTC	720
Leu	Glu	Ile	Asp	Asn	Arg	Gln	Cys	Val	Gln	Ala	Ser	Ser	Gln	Cys	Phe	
225					230					235					240	
CAG	AGT	GCC	ACC	GAC	GTG	GCC	GCA	TTC	CTG	GGA	GCG	CTC	GCC	TCG	CTG	768
Gln	Ser	Ala	Thr	Asp	Val	Ala	Ala	Phe	Leu	Gly	Ala	Leu	Ala	Ser	Leu	
				245					250					255		
GGC	AGC	CTC	AAC	ATC	CCC	TAC	AAG	ATC	GAG	GCC	GTG	CAG	AGT	GAG	ACC	816
Gly	Ser	Leu	Asn	Ile	Pro	Tyr	Lys	Ile	Glu	Ala	Val	Gln	Ser	Glu	Thr	
			260					265					270			
GTG	GAG	CCG	CCC	CCG	CCG	GCG	CAG	CTG	CAC	TTC	ATG	TAC	GTG	GCG	GCG	864
Val	Glu	Pro	Pro	Pro	Pro	Ala	Gln	Leu	His	Phe	Met	Tyr	Val	Ala	Ala	
		275					280					285				
GCC	GCC	TTT	GTG	CTT	CTG	TTC	TTC	GTG	GGC	TGC	GGG	GTG	CTG	CTG	TCC	912
Ala	Ala	Phe	Val	Leu	Leu	Phe	Phe	Val	Gly	Cys	Gly	Val	Leu	Leu	Ser	
		290				295					300					
CGC	AAG	CGC	CGG	CGG	CAG	CAT	GGC	CAG	CTC	TGG	TTC	CCT	GAG	GGC	TTC	960
Arg	Lys	Arg	Arg	Arg	Gln	His	Gly	Gln	Leu	Trp	Phe	Pro	Glu	Gly	Phe	
305					310					315					320	
AAA	GTG	TCT	GAG	GCC	AGC	AAG	AAG	AAG	CGG	CGG	GAG	CCC	CTC	GGC	GAG	1008
Lys	Val	Ser	Glu	Ala	Ser	Lys	Lys	Lys	Arg	Arg	Glu	Pro	Leu	Gly	Glu	
				325					330					335		

-102-

GAC TCC GTG GGC CTC AAG CCC CTG AAG AAC GCT TCA GAC GGT GCC CTC Asp Ser Val Gly Leu Lys Pro Leu Lys Asn Ala Ser Asp Gly Ala Leu 340 345 350	1056
ATG GAC GAC AAC CAG AAT GAG TGG GGG GAC GAG GAC CTG GAG ACC AAG Met Asp Asp Asn Gln Asn Glu Trp Gly Asp Glu Asp Leu Glu Thr Lys 355 360 365	1104
AAG TTC CGG TTC GAG GAG CCC GTG GTT CTG CCT GAC CTG GAC GAC CAG Lys Phe Arg Phe Glu Glu Pro Val Val Leu Pro Asp Leu Asp Asp Gln 370 375 380	1152
ACA GAC CAC CGG CAG TGG ACT CAG CAG CAC CTG GAT GCC GCT GAC CTG Thr Asp His Arg Gln Trp Thr Gln Gln His Leu Asp Ala Ala Asp Leu 385 390 395 400	1200
CGC ATG TCT GCC ATG GCC CCC ACA CCG CCC CAG GGT GAG GTT GAC GCC Arg Met Ser Ala Met Ala Pro Thr Pro Pro Gln Gly Glu Val Asp Ala 405 410 415	1248
GAC TGC ATG GAC GTC AAT GTC CGC GGG CCT GAT GGC TTC ACC CCG CTC Asp Cys Met Asp Val Asn Val Arg Gly Pro Asp Gly Phe Thr Pro Leu 420 425 430	1296
ATG ATC GCC TCC TGC AGC GGG GGC GGC CTG GAG ACG GGC AAC AGC GAG Met Ile Ala Ser Cys Ser Gly Gly Gly Leu Glu Thr Gly Asn Ser Glu 435 440 445	1344
GAA GAG GAG GAC GCG CCG GCC GTC ATC TCC GAC TTC ATC TAC CAG GGC Glu Glu Glu Asp Ala Pro Ala Val Ile Ser Asp Phe Ile Tyr Gln Gly 450 455 460	1392
GCC AGC CTG CAC AAC CAG ACA GAC CGC ACG GGC GAG ACC GCC TTG CAC Ala Ser Leu His Asn Gln Thr Asp Arg Thr Gly Glu Thr Ala Leu His 465 470 475 480	1440
CTG GCC GCC CGC TAC TCA CGC TCT GAT GCC GCC AAG CGC CTG CTG GAG Leu Ala Ala Arg Tyr Ser Arg Ser Asp Ala Ala Lys Arg Leu Leu Glu 485 490 495	1488
GCC AGC GCA GAT GCC AAC ATC CAG GAC AAC ATG GGC CGC ACC CCG CTG Ala Ser Ala Asp Ala Asn Ile Gln Asp Asn Met Gly Arg Thr Pro Leu 500 505 510	1536
CAT GCG GCT GTG TCT GCC GAC GCA CAA GGT GTC TTC CAG ATC CTG ATC His Ala Ala Val Ser Ala Asp Ala Gln Gly Val Phe Gln Ile Leu Ile 515 520 525	1584
CGG AAC CGA GCC ACA GAC CTG GAT GCC CGC ATG CAT GAT GGC ACG ACG Arg Asn Arg Ala Thr Asp Leu Asp Ala Arg Met His Asp Gly Thr Thr 530 535 540	1632
CCA CTG ATC CTG GCT GCC CGC CTG GCC GTG GAG GGC ATG CTG GAG GAC Pro Leu Ile Leu Ala Ala Arg Leu Ala Val Glu Gly Met Leu Glu Asp 545 550 555 560	1680
CTC ATC AAC TCA CAC GCC GAC GTC AAC GCC GTA GAT GAC CTG GGC AAG Leu Ile Asn Ser His Ala Asp Val Asn Ala Val Asp Asp Leu Gly Lys 565 570 575	1728
TCC GCC CTG CAC TGG GCC GCC GCC GTG AAC AAT GTG GAT GCC GCA GTT Ser Ala Leu His Trp Ala Ala Ala Val Asn Asn Val Asp Ala Ala Val 580 585 590	1776
GTG CTC CTG AAG AAC GGG GCT AAC AAA GAT ATG CAG AAC AAC AGG GAG Val Leu Leu Lys Asn Gly Ala Asn Lys Asp Met Gln Asn Asn Arg Glu 595 600 605	1824

-103-

GAG ACA CCC CTG TTT CTG GCC GCC CGG GAG GGC AGC TAC GAG ACC GCC Glu Thr Pro Leu Phe Leu Ala Ala Arg Glu Gly Ser Tyr Glu Thr Ala 610 615 620	1872
AAG GTG CTG CTG GAC CAC TTT GCC AAC CGG GAC ATC ACG GAT CAT ATG Lys Val Leu Leu Asp His Phe Ala Asn Arg Asp Ile Thr Asp His Met 625 630 635 640	1920
GAC CGC CTG CCG CGC GAC ATC GCA CAG GAG CGC ATG CAT CAC GAC ATC Asp Arg Leu Pro Arg Asp Ile Ala Gln Glu Arg Met His His Asp Ile 645 650 655	1968
GTG AGG CTG CTG GAC GAG TAC AAC CTG GTG CGC AGC CCG CAG CTG CAC Val Arg Leu Leu Asp Glu Tyr Asn Leu Val Arg Ser Pro Gln Leu His 660 665 670	2016
GGA GCC CCG CTG GGG GGC ACG CCC ACC CTG TCG CCC CCG CTC TGC TCG Gly Ala Pro Leu Gly Gly Thr Pro Thr Leu Ser Pro Pro Leu Cys Ser 675 680 685	2064
CCC AAC GGC TAC CTG GGC AGC CTC AAG CCC GGC GTG CAG GGC AAG AAG Pro Asn Gly Tyr Leu Gly Ser Leu Lys Pro Gly Val Gln Gly Lys Lys 690 695 700	2112
GTC CGC AAG CCC AGC AGC AAA GGC CTG GCC TGT GGA AGC AAG GAG GCC Val Arg Lys Pro Ser Ser Lys Gly Leu Ala Cys Gly Ser Lys Glu Ala 705 710 715 720	2160
AAG GAC CTC AAG GCA CGG AGG AAG AAG TCC CAG GAT GGC AAG GGC TGC Lys Asp Leu Lys Ala Arg Arg Lys Lys Ser Gln Asp Gly Lys Gly Cys 725 730 735	2208
CTG CTG GAC AGC TCC GGC ATG CTC TCG CCC GTG GAC TCC CTG GAG TCA Leu Leu Asp Ser Ser Gly Met Leu Ser Pro Val Asp Ser Leu Glu Ser 740 745 750	2256
CCC CAT GGC TAC CTG TCA GAC GTG GCC TCG CCG CCA CTG CTG CCC TCC Pro His Gly Tyr Leu Ser Asp Val Ala Ser Pro Pro Leu Leu Pro Ser 755 760 765	2304
CCG TTC CAG CAG TCT CCG TCC GTG CCC CTC AAC CAC CTG CCT GGG ATG Pro Phe Gln Gln Ser Pro Ser Val Pro Leu Asn His Leu Pro Gly Met 770 775 780	2352
CCC GAC ACC CAC CTG GGC ATC GGG CAC CTG AAC GTG GCG GCC AAG CCC Pro Asp Thr His Leu Gly Ile Gly His Leu Asn Val Ala Ala Lys Pro 785 790 795 800	2400
GAG ATG GCG GCG CTG GGT GGG GGC GGC CGG CTG GCC TTT GAG ACT GGC Glu Met Ala Ala Leu Gly Gly Gly Gly Arg Leu Ala Phe Glu Thr Gly 805 810 815	2448
CCA CCT CGT CTC TCC CAC CTG CCT GTG GCC TCT GGC ACC AGC ACC GTC Pro Pro Arg Leu Ser His Leu Pro Val Ala Ser Gly Thr Ser Thr Val 820 825 830	2496
CTG GGC TCC AGC AGC GGA GGG GCC CTG AAT TTC ACT GTG GGC GGG TCC Leu Gly Ser Ser Ser Gly Gly Ala Leu Asn Phe Thr Val Gly Gly Ser 835 840 845	2544
ACC AGT TTG AAT GGT CAA TGC GAG TGG CTG TCC CGG CTG CAG AGC GGC Thr Ser Leu Asn Gly Gln Cys Glu Trp Leu Ser Arg Leu Gln Ser Gly 850 855 860	2592
ATG GTG CCG AAC CAA TAC AAC CCT CTG CGG GGG AGT GTG GCA CCA GGC Met Val Pro Asn Gln Tyr Asn Pro Leu Arg Gly Ser Val Ala Pro Gly 865 870 875 880	2640

-104-

CCC CTG AGC ACA CAG GCC CCC TCC CTG CAG CAT GGC ATG GTA GGC CCG Pro Leu Ser Thr Gln Ala Pro Ser Leu Gln His Gly Met Val Gly Pro 885 890 895	2688
CTG CAC AGT AGC CTT GCT GCC AGC GCC CTG TCC CAG ATG ATG AGC TAC Leu His Ser Ser Leu Ala Ala Ser Ala Leu Ser Gln Met Met Ser Tyr 900 905 910	2736
CAG GGC CTG CCC AGC ACC CGG CTG GCC ACC CAG CCT CAC CTG GTG CAG Gln Gly Leu Pro Ser Thr Arg Leu Ala Thr Gln Pro His Leu Val Gln 915 920 925	2784
ACC CAG CAG GTG CAG CCA CAA AAC TTA CAG ATG CAG CAG CAG AAC CTG Thr Gln Gln Val Gln Pro Gln Asn Leu Gln Met Gln Gln Gln Asn Leu 930 935 940	2832
CAG CCA GCA AAC ATC CAG CAG CAG CAA AGC CTG CAG CCG CCA CCA CCA Gln Pro Ala Asn Ile Gln Gln Gln Gln Ser Leu Gln Pro Pro Pro Pro 945 950 955 960	2880
CCA CCA CAG CCG CAC CTT GGC GTG AGC TCA GCA GCC AGC GGC CAC CTG Pro Pro Gln Pro His Leu Gly Val Ser Ser Ala Ala Ser Gly His Leu 965 970 975	2928
GGC CGG AGC TTC CTG AGT GGA GAG CCG AGC CAG GCA GAC GTG CAG CCA Gly Arg Ser Phe Leu Ser Gly Glu Pro Ser Gln Ala Asp Val Gln Pro 980 985 990	2976
CTG GGC CCC AGC AGC CTG GCG GTG CAC ACT ATT CTG CCC CAG GAG AGC Leu Gly Pro Ser Ser Leu Ala Val His Thr Ile Leu Pro Gln Glu Ser 995 1000 1005	3024
CCC GCC CTG CCC ACG TCG CTG CCA TCC TCG CTG GTC CCA CCC GTG ACC Pro Ala Leu Pro Thr Ser Leu Pro Ser Ser Leu Val Pro Pro Val Thr 1010 1015 1020	3072
GCA GCC CAG TTC CTG ACG CCC CCC TCG CAG CAC AGC TAC TCC TCG CCT Ala Ala Gln Phe Leu Thr Pro Pro Ser Gln His Ser Tyr Ser Ser Pro 1025 1030 1035 1040	3120
GTG GAC AAC ACC CCC AGC CAC CAG CTA CAG GTG CCT GTT CCT GTA ATG Val Asp Asn Thr Pro Ser His Gln Leu Gln Val Pro Val Pro Val Met 1045 1050 1055	3168
GTA ATG ATC CGA TCT TCG GAT CCT TCT AAA GGC TCA TCA ATT TTG ATC Val Met Ile Arg Ser Ser Asp Pro Ser Lys Gly Ser Ser Ile Leu Ile 1060 1065 1070	3216
GAA GCT CCC GAC TCA TGG Glu Ala Pro Asp Ser Trp 1075	3234

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1078 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Gln Glu Asp Ala Gly Asn Lys Val Cys Ser Leu Gln Cys Asn Asn
1 5 10 15

-105-

His Ala Cys Gly Trp Asp Gly Gly Asp Cys Ser Leu Asn Phe Asn Asp
 20 25 30
 Pro Trp Lys Asn Cys Thr Gln Ser Leu Gln Cys Trp Lys Tyr Phe Ser
 35 40 45
 Asp Gly His Cys Asp Ser Gln Cys Asn Ser Ala Gly Cys Leu Phe Asp
 50 55 60
 Gly Phe Asp Cys Gln Arg Ala Glu Gly Gln Cys Asn Pro Leu Tyr Asp
 65 70 75 80
 Gln Tyr Cys Lys Asp His Phe Ser Asp Gly His Cys Asp Gln Gly Cys
 85 90 95
 Asn Ser Ala Glu Cys Glu Trp Asp Gly Leu Asp Cys Ala Glu His Val
 100 105 110
 Pro Glu Arg Leu Ala Ala Gly Thr Leu Val Val Val Val Leu Met Pro
 115 120 125
 Pro Glu Gln Leu Arg Asn Ser Ser Phe His Phe Leu Arg Glu Leu Ser
 130 135 140
 Arg Val Leu His Thr Asn Val Val Phe Lys Arg Asp Ala His Gly Gln
 145 150 155 160
 Gln Met Ile Phe Pro Tyr Tyr Gly Arg Glu Glu Glu Leu Arg Lys His
 165 170 175
 Pro Ile Lys Arg Ala Ala Glu Gly Trp Ala Ala Pro Asp Ala Leu Leu
 180 185 190
 Gly Gln Val Lys Ala Ser Leu Leu Pro Gly Gly Ser Glu Gly Gly Arg
 195 200 205
 Arg Arg Arg Glu Leu Asp Pro Met Asp Val Arg Gly Ser Ile Val Tyr
 210 215 220
 Leu Glu Ile Asp Asn Arg Gln Cys Val Gln Ala Ser Ser Gln Cys Phe
 225 230 235 240
 Gln Ser Ala Thr Asp Val Ala Ala Phe Leu Gly Ala Leu Ala Ser Leu
 245 250 255
 Gly Ser Leu Asn Ile Pro Tyr Lys Ile Glu Ala Val Gln Ser Glu Thr
 260 265 270
 Val Glu Pro Pro Pro Pro Ala Gln Leu His Phe Met Tyr Val Ala Ala
 275 280 285
 Ala Ala Phe Val Leu Leu Phe Phe Val Gly Cys Gly Val Leu Leu Ser
 290 295 300
 Arg Lys Arg Arg Arg Gln His Gly Gln Leu Trp Phe Pro Glu Gly Phe
 305 310 315 320
 Lys Val Ser Glu Ala Ser Lys Lys Lys Arg Arg Glu Pro Leu Gly Glu
 325 330 335
 Asp Ser Val Gly Leu Lys Pro Leu Lys Asn Ala Ser Asp Gly Ala Leu
 340 345 350
 Met Asp Asp Asn Gln Asn Glu Trp Gly Asp Glu Asp Leu Glu Thr Lys
 355 360 365
 Lys Phe Arg Phe Glu Glu Pro Val Val Leu Pro Asp Leu Asp Asp Gln

-106-

370 375 380
 Thr Asp His Arg Gln Trp Thr Gln Gln His Leu Asp Ala Ala Asp Leu
 385 390 395 400
 Arg Met Ser Ala Met Ala Pro Thr Pro Pro Gln Gly Glu Val Asp Ala
 405 410 415
 Asp Cys Met Asp Val Asn Val Arg Gly Pro Asp Gly Phe Thr Pro Leu
 420 425 430
 Met Ile Ala Ser Cys Ser Gly Gly Gly Leu Glu Thr Gly Asn Ser Glu
 435 440 445
 Glu Glu Glu Asp Ala Pro Ala Val Ile Ser Asp Phe Ile Tyr Gln Gly
 450 455 460
 Ala Ser Leu His Asn Gln Thr Asp Arg Thr Gly Glu Thr Ala Leu His
 465 470 475 480
 Leu Ala Ala Arg Tyr Ser Arg Ser Asp Ala Ala Lys Arg Leu Leu Glu
 485 490 495
 Ala Ser Ala Asp Ala Asn Ile Gln Asp Asn Met Gly Arg Thr Pro Leu
 500 505 510
 His Ala Ala Val Ser Ala Asp Ala Gln Gly Val Phe Gln Ile Leu Ile
 515 520 525
 Arg Asn Arg Ala Thr Asp Leu Asp Ala Arg Met His Asp Gly Thr Thr
 530 535 540
 Pro Leu Ile Leu Ala Ala Arg Leu Ala Val Glu Gly Met Leu Glu Asp
 545 550 555 560
 Leu Ile Asn Ser His Ala Asp Val Asn Ala Val Asp Asp Leu Gly Lys
 565 570 575
 Ser Ala Leu His Trp Ala Ala Ala Val Asn Asn Val Asp Ala Ala Val
 580 585 590
 Val Leu Leu Lys Asn Gly Ala Asn Lys Asp Met Gln Asn Asn Arg Glu
 595 600 605
 Glu Thr Pro Leu Phe Leu Ala Ala Arg Glu Gly Ser Tyr Glu Thr Ala
 610 615 620
 Lys Val Leu Leu Asp His Phe Ala Asn Arg Asp Ile Thr Asp His Met
 625 630 635 640
 Asp Arg Leu Pro Arg Asp Ile Ala Gln Glu Arg Met His His Asp Ile
 645 650 655
 Val Arg Leu Leu Asp Glu Tyr Asn Leu Val Arg Ser Pro Gln Leu His
 660 665 670
 Gly Ala Pro Leu Gly Gly Thr Pro Thr Leu Ser Pro Pro Leu Cys Ser
 675 680 685
 Pro Asn Gly Tyr Leu Gly Ser Leu Lys Pro Gly Val Gln Gly Lys Lys
 690 695 700
 Val Arg Lys Pro Ser Ser Lys Gly Leu Ala Cys Gly Ser Lys Glu Ala
 705 710 715 720
 Lys Asp Leu Lys Ala Arg Arg Lys Lys Ser Gln Asp Gly Lys Gly Cys
 725 730 735

-107-

Leu Leu Asp Ser Ser Gly Met Leu Ser Pro Val Asp Ser Leu Glu Ser
 740 745 750
 Pro His Gly Tyr Leu Ser Asp Val Ala Ser Pro Pro Leu Leu Pro Ser
 755 760 765
 Pro Phe Gln Gln Ser Pro Ser Val Pro Leu Asn His Leu Pro Gly Met
 770 775 780
 Pro Asp Thr His Leu Gly Ile Gly His Leu Asn Val Ala Ala Lys Pro
 785 790 795 800
 Glu Met Ala Ala Leu Gly Gly Gly Gly Arg Leu Ala Phe Glu Thr Gly
 805 810 815
 Pro Pro Arg Leu Ser His Leu Pro Val Ala Ser Gly Thr Ser Thr Val
 820 825 830
 Leu Gly Ser Ser Ser Gly Gly Ala Leu Asn Phe Thr Val Gly Gly Ser
 835 840 845
 Thr Ser Leu Asn Gly Gln Cys Glu Trp Leu Ser Arg Leu Gln Ser Gly
 850 855 860
 Met Val Pro Asn Gln Tyr Asn Pro Leu Arg Gly Ser Val Ala Pro Gly
 865 870 875 880
 Pro Leu Ser Thr Gln Ala Pro Ser Leu Gln His Gly Met Val Gly Pro
 885 890 895
 Leu His Ser Ser Leu Ala Ala Ser Ala Leu Ser Gln Met Met Ser Tyr
 900 905 910
 Gln Gly Leu Pro Ser Thr Arg Leu Ala Thr Gln Pro His Leu Val Gln
 915 920 925
 Thr Gln Gln Val Gln Pro Gln Asn Leu Gln Met Gln Gln Gln Asn Leu
 930 935 940
 Gln Pro Ala Asn Ile Gln Gln Gln Gln Ser Leu Gln Pro Pro Pro Pro
 945 950 955 960
 Pro Pro Gln Pro His Leu Gly Val Ser Ser Ala Ala Ser Gly His Leu
 965 970 975
 Gly Arg Ser Phe Leu Ser Gly Glu Pro Ser Gln Ala Asp Val Gln Pro
 980 985 990
 Leu Gly Pro Ser Ser Leu Ala Val His Thr Ile Leu Pro Gln Glu Ser
 995 1000 1005
 Pro Ala Leu Pro Thr Ser Leu Pro Ser Ser Leu Val Pro Pro Val Thr
 1010 1015 1020
 Ala Ala Gln Phe Leu Thr Pro Pro Ser Gln His Ser Tyr Ser Ser Pro
 1025 1030 1035 1040
 Val Asp Asn Thr Pro Ser His Gln Leu Gln Val Pro Val Pro Val Met
 1045 1050 1055
 Val Met Ile Arg Ser Ser Asp Pro Ser Lys Gly Ser Ser Ile Leu Ile
 1060 1065 1070
 Glu Ala Pro Asp Ser Trp
 1075

(2) INFORMATION FOR SEQ ID NO:12:

-108-

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4268 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 2..1972

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

G GAG GTG GAT GTG TTA GAT GTG AAT GTC CGT GGC CCA GAT GGC TGC	46
Glu Val Asp Val Leu Asp Val Asn Val Arg Gly Pro Asp Gly Cys	
1 5 10 15	
ACC CCA TTG ATG TTG GCT TCT CTC CGA GGA GGC AGC TCA GAT TTG AGT	94
Thr Pro Leu Met Leu Ala Ser Leu Arg Gly Gly Ser Ser Asp Leu Ser	
20 25 30	
GAT GAA GAT GAA GAT GCA GAG GAC TCT TCT GCT AAC ATC ATC ACA GAC	142
Asp Glu Asp Glu Asp Ala Glu Asp Ser Ser Ala Asn Ile Ile Thr Asp	
35 40 45	
TTG GTC TAC CAG GGT GCC AGC CTC CAG GCC CAG ACA GAC CGG ACT GGT	190
Leu Val Tyr Gln Gly Ala Ser Leu Gln Ala Gln Thr Asp Arg Thr Gly	
50 55 60	
GAG ATG GCC CTG CAC CTT GCA GCC CGC TAC TCA CGG GCT GAT GCT GCC	238
Glu Met Ala Leu His Leu Ala Ala Arg Tyr Ser Arg Ala Asp Ala Ala	
65 70 75	
AAG CGT CTC CTG GAT GCA GGT GCA GAT GCC AAT GCC CAG GAC AAC ATG	286
Lys Arg Leu Leu Asp Ala Gly Ala Asp Ala Asn Ala Gln Asp Asn Met	
80 85 90 95	
GGC CGC TGT CCA CTC CAT GCT GCA GTG GCA GCT GAT GCC CAA GGT GTC	334
Gly Arg Cys Pro Leu His Ala Ala Val Ala Ala Asp Ala Gln Gly Val	
100 105 110	
TTC CAG ATT CTG ATT CGC AAC CGA GTA ACT GAT CTA GAT GCC AGG ATG	382
Phe Gln Ile Leu Ile Arg Asn Arg Val Thr Asp Leu Asp Ala Arg Met	
115 120 125	
AAT GAT GGT ACT ACA CCC CTG ATC CTG GCT GCC CGC CTG GCT GTG GAG	430
Asn Asp Gly Thr Thr Pro Leu Ile Leu Ala Ala Arg Leu Ala Val Glu	
130 135 140	
GGA ATG GTG GCA GAA CTG ATC AAC TGC CAA GCG GAT GTG AAT GCA GTG	478
Gly Met Val Ala Glu Leu Ile Asn Cys Gln Ala Asp Val Asn Ala Val	
145 150 155	
GAT GAC CAT GGA AAA TCT GCT CTT CAC TGG GCA GCT GCT GTC AAT AAT	526
Asp Asp His Gly Lys Ser Ala Leu His Trp Ala Ala Ala Val Asn Asn	
160 165 170 175	
GTG GAG GCA ACT CTT TTG TTG TTG AAA AAT GGG GCC AAC CGA GAC ATG	574
Val Glu Ala Thr Leu Leu Leu Lys Asn Gly Ala Asn Arg Asp Met	
180 185 190	
CAG GAC AAC AAG GAA GAG ACA CCT CTG TTT CTT GCT GCC CGG GAG GGG	622
Gln Asp Asn Lys Glu Glu Thr Pro Leu Phe Leu Ala Ala Arg Glu Gly	
195 200 205	

AGC	TAT	GAA	GCA	GCC	AAG	ATC	CTG	TTA	GAC	CAT	TTT	GCC	AAT	CGA	GAC	670
Ser	Tyr	Glu	Ala	Ala	Lys	Ile	Leu	Leu	Asp	His	Phe	Ala	Asn	Arg	Asp	
		210					215					220				
ATC	ACA	GAC	CAT	ATG	GAT	CGT	CTT	CCC	CGG	GAT	GTG	GCT	CGG	GAT	CGC	718
Ile	Thr	Asp	His	Met	Asp	Arg	Leu	Pro	Arg	Asp	Val	Ala	Arg	Asp	Arg	
		225				230					235					
ATG	CAC	CAT	GAC	ATT	GTG	CGC	CTT	CTG	GAT	GAA	TAC	AAT	GTG	ACC	CCA	766
Met	His	His	Asp	Ile	Val	Arg	Leu	Leu	Asp	Glu	Tyr	Asn	Val	Thr	Pro	
240					245					250					255	
AGC	CCT	CCA	GGC	ACC	GTG	TTG	ACT	TCT	GCT	CTC	TCA	CCT	GTC	ATC	TGT	814
Ser	Pro	Pro	Gly	Thr	Val	Leu	Thr	Ser	Ala	Leu	Ser	Pro	Val	Ile	Cys	
				260					265					270		
GGG	CCC	AAC	AGA	TCT	TTC	CTC	AGC	CTG	AAG	CAC	ACC	CCA	ATG	GGC	AAG	862
Gly	Pro	Asn	Arg	Ser	Phe	Leu	Ser	Leu	Lys	His	Thr	Pro	Met	Gly	Lys	
			275					280					285			
AAG	TCT	AGA	CGG	CCC	AGT	GCC	AAG	AGT	ACC	ATG	CCT	ACT	AGC	CTC	CCT	910
Lys	Ser	Arg	Arg	Pro	Ser	Ala	Lys	Ser	Thr	Met	Pro	Thr	Ser	Leu	Pro	
		290					295					300				
AAC	CTT	GCC	AAG	GAG	GCA	AAG	GAT	GCC	AAG	GGT	AGT	AGG	AGG	AAG	AAG	958
Asn	Leu	Ala	Lys	Glu	Ala	Lys	Asp	Ala	Lys	Gly	Ser	Arg	Arg	Lys	Lys	
		305				310					315					
TCT	CTG	AGT	GAG	AAG	GTC	CAA	CTG	TCT	GAG	AGT	TCA	GTA	ACT	TTA	TCC	1006
Ser	Leu	Ser	Glu	Lys	Val	Gln	Leu	Ser	Glu	Ser	Ser	Val	Thr	Leu	Ser	
320					325					330					335	
CCT	GTT	GAT	TCC	CTA	GAA	TCT	CCT	CAC	ACG	TAT	GTT	TCC	GAC	ACC	ACA	1054
Pro	Val	Asp	Ser	Leu	Glu	Ser	Pro	His	Thr	Tyr	Val	Ser	Asp	Thr	Thr	
				340					345					350		
TCC	TCT	CCA	ATG	ATT	ACA	TCC	CCT	GGG	ATC	TTA	CAG	GCC	TCA	CCC	AAC	1102
Ser	Ser	Pro	Met	Ile	Thr	Ser	Pro	Gly	Ile	Leu	Gln	Ala	Ser	Pro	Asn	
			355					360					365			
CCT	ATG	TTG	GCC	ACT	GCC	GCC	CCT	CCT	GCC	CCA	GTC	CAT	GCC	CAG	CAT	1150
Pro	Met	Leu	Ala	Thr	Ala	Ala	Pro	Pro	Ala	Pro	Val	His	Ala	Gln	His	
		370					375					380				
GCA	CTA	TCT	TTT	TCT	AAC	CTT	CAT	GAA	ATG	CAG	CCT	TTG	GCA	CAT	GGG	1198
Ala	Leu	Ser	Phe	Ser	Asn	Leu	His	Glu	Met	Gln	Pro	Leu	Ala	His	Gly	
		385				390					395					
GCC	AGC	ACT	GTG	CTT	CCC	TCA	GTG	AGC	CAG	TTG	CTA	TCC	CAC	CAC	CAC	1246
Ala	Ser	Thr	Val	Leu	Pro	Ser	Val	Ser	Gln	Leu	Leu	Ser	His	His	His	
400					405					410					415	
ATT	GTG	TCT	CCA	GGC	AGT	GGC	AGT	GCT	GGA	AGC	TTG	AGT	AGG	CTC	CAT	1294
Ile	Val	Ser	Pro	Gly	Ser	Gly	Ser	Ala	Gly	Ser	Leu	Ser	Arg	Leu	His	
				420					425					430		
CCA	GTC	CCA	GTC	CCA	GCA	GAT	TGG	ATG	AAC	CGC	ATG	GAG	GTG	AAT	GAG	1342
Pro	Val	Pro	Val	Pro	Ala	Asp	Trp	Met	Asn	Arg	Met	Glu	Val	Asn	Glu	
			435				440						445			
ACC	CAG	TAC	AAT	GAG	ATG	TTT	GGT	ATG	GTC	CTG	GCT	CCA	GCT	GAG	GGC	1390
Thr	Gln	Tyr	Asn	Glu	Met	Phe	Gly	Met	Val	Leu	Ala	Pro	Ala	Glu	Gly	
		450					455					460				
ACC	CAT	CCT	GGC	ATA	GCT	CCC	CAG	AGC	AGG	CCA	CCT	GAA	GGG	AAG	CAC	1438
Thr	His	Pro	Gly	Ile	Ala	Pro	Gln	Ser	Arg	Pro	Pro	Glu	Gly	Lys	His	
		465				470					475					

-110-

ATA ACC ACC CCT CGG GAG CCC TTG CCC CCC ATT GTG ACT TTC CAG CTC Ile Thr Thr Pro Arg Glu Pro Leu Pro Pro Ile Val Thr Phe Gln Leu 480 485 490 495	1486
ATC CCT AAA GGC AGT ATT GCC CAA CCA GCG GGG GCT CCC CAG CCT CAG Ile Pro Lys Gly Ser Ile Ala Gln Pro Ala Gly Ala Pro Gln Pro Gln 500 505 510	1534
TCC ACC TGC CCT CCA GCT GTT GCG GGC CCC CTG CCC ACC ATG TAC CAG Ser Thr Cys Pro Pro Ala Val Ala Gly Pro Leu Pro Thr Met Tyr Gln 515 520 525	1582
ATT CCA GAA ATG GCC CGT TTG CCC AGT GTG GCT TTC CCC ACT GCC ATG Ile Pro Glu Met Ala Arg Leu Pro Ser Val Ala Phe Pro Thr Ala Met 530 535 540	1630
ATG CCC CAG CAG GAC GGG CAG GTA GCT CAG ACC ATT CTC CCA GCC TAT Met Pro Gln Gln Asp Gly Gln Val Ala Gln Thr Ile Leu Pro Ala Tyr 545 550 555	1678
CAT CCT TTC CCA GCC TCT GTG GGC AAG TAC CCC ACA CCC CCT TCA CAG His Pro Phe Pro Ala Ser Val Gly Lys Tyr Pro Thr Pro Pro Ser Gln 560 565 570 575	1726
CAC AGT TAT GCT TCC TCA AAT GCT GCT GAG CGA ACA CCC AGT CAC AGT His Ser Tyr Ala Ser Ser Asn Ala Ala Glu Arg Thr Pro Ser His Ser 580 585 590	1774
GGT CAC CTC CAG GGT GAG CAT CCC TAC CTG ACA CCA TCC CCA GAG TCT Gly His Leu Gln Gly Glu His Pro Tyr Leu Thr Pro Ser Pro Glu Ser 595 600 605	1822
CCT GAC CAG TGG TCA AGT TCA TCA CCC CAC TCT GCT TCT GAC TGG TCA Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser Ala Ser Asp Trp Ser 610 615 620	1870
GAT GTG ACC ACC AGC CCT ACC CCT GGG GGT GCT GGA GGA GGT CAG CGG Asp Val Thr Thr Ser Pro Thr Pro Gly Gly Ala Gly Gly Gly Gln Arg 625 630 635	1918
GGA CCT GGG ACA CAC ATG TCT GAG CCA CCA CAC AAC AAC ATG CAG GTT Gly Pro Gly Thr His Met Ser Glu Pro Pro His Asn Asn Met Gln Val 640 645 650 655	1966
TAT GCG TGAGAGAGTC CACCTCCAGT GTAGAGACAT AACTGACTTT TGTAATGCT Tyr Ala	2022
GCTGAGGAAC AAATGAAGGT CATCCGGGAG AGAAATGAAG AAATCTCTGG AGCCAGCTTC	2082
TAGAGGTAGG AAAGAGAAGA TGTTCTTATT CAGATAATGC AAGAGAAGCA ATTCGTCAGT	2142
TTCACTGGGT ATCTGCAAGG CTTATTGATT ATTCTAATCT AATAAGACAA GTTTGTGGAA	2202
ATGCAAGATG AATACAAGCC TTGGGTCCAT GTTACTCTC TTCTATTTGG AGAATAAGAT	2262
GGATGCTTAT TGAAGCCCAG ACATTCTTGC AGCTTGGACT GCATTTTAAG CCCTGCAGGC	2322
TTCTGCCATA TCCATGAGAA GATTCTACAC TAGCGTCCTG TTGGGAATTA TGCCCTGGAA	2382
TTCTGCCTGA ATTGACCTAC GCATCTCCTC CTCCTTGGAC ATTCTTTTGT CTTCAATTTGG	2442
TGCTTTTGGT TTTGCACCTC TCCGTGATTG TAGCCCTACC AGCATGTTAT AGGGCAAGAC	2502
CTTTGTGCTT TTGATCATTC TGGCCCATGA AAGCAACTTT GGTCTCCTTT CCCCTCCTGT	2562
CTTCCCGGTA TCCCTTGGAG TCTCACAAGG TTTACTTTGG TATGGTTCTC AGCACAAACC	2622

-111-

TTTCAAGTAT GTTGTCTTCTT TGGAAAATGG ACATACTGTA TTGTGTTCTC CTGCATATAT	2682
CATTCCTGGA GAGAGAAGGG GAGAAGAATA CTTTCTTCA ACAAATTTTG GGGGCAGGAG	2742
ATCCCTTCAA GAGGCTGCAC CTTAATTTTT CTTGTCTGTG TGCAGGTCTT CATATAAACT	2802
TTACCAGGAA GAAGGGTGTG AGTTTGTGTG TTTTCTGTGT ATGGGCCTGG TCAGTGTAAG	2862
GTTTTATCCT TGATAGTCTA GTTACTATGA CCCTCCCCAC TTTTTTAAAA CCAGAAAAAG	2922
GTTTGGAATG TTGGAATGAC CAAGAGACAA GTTAACTCGT GCAAGAGCCA GTTACCCACC	2982
CACAGGTCCC CCTACTTCCT GCCAAGCATT CCATTGACTG CCTGTATGGA ACACATTTGT	3042
CCCAGATCTG AGCATTCTAG GCCTGTTTCA CTCCTCACC CAGCATATGA AACTAGTCTT	3102
AACTGTTGAG CCTTTCCTTT CATATCCACA GAAGACACTG TCTCAAATGT TGTACCCTTG	3162
CCATTTAGGA CTGAACTTTC CTTAGCCCAA GGGACCCAGT GACAGTTGTC TTCCGTTTGT	3222
CAGATGATCA GTCTCTACTG ATTATCTTGC TGCTTAAAGG CCTGCTCACC AATCTTTCTT	3282
TCACACCGTG TGGTCCGTGT TACTGGTATA CCCAGTATGT TCTCACTGAA GACATGGACT	3342
TTATATGTTT AAGTGCAGGA ATTGGAAAAG TGGACTTGTT TTCTATGATC CAAAACAGCC	3402
CTATAAGAAG GTTGGAAAAG GAGGAACTAT ATAGCAGCCT TTGCTATTTT CTGCTACCAT	3462
TTCTTTTCCT CTGAAGCGGC CATGACATTC CCTTTGGCAA CTAACGTAGA AACTCAACAG	3522
AACATTTTCC TTTCTAGAG TCACCTTTTA GATGATAATG GACAACTATA GACTTGCTCA	3582
TTGTTGAGAC TGATTGCCCC TCACCTGAAT CCACTCTCTG TATTCATGCT CTTGGCAATT	3642
TCTTTGACTT TCTTTTAAGG GCAGAAGCAT TTTAGTTAAT TGTAAGATAA GAATAGTTTT	3702
CTTCTCTTC TCCTTGGGCC AGTTAATAAT TGGTCCATGG CTACACTGCA ACTTCCGTCC	3762
AGTGCTGTGA TGCCCATGAC ACCTGCAAAA TAAGTTCTGC CTGGGCATTT TGTAGATATT	3822
AACAGGTGAA TTCCCGACTC TTTTGGTTTG AATGACAGTT CTCATTCCTT CTATGGCTGC	3882
AAGTATGCAT CAGTGCTTCC CACTTACCTG ATTTGTCTGT CGGTGGCCCC ATATGGAAAC	3942
CCTGCGTGTC TGTTGGCATA ATAGTTTACA AATGGTTTTT TCAGTCCTAT CCAAATTTAT	4002
TGAACCAACA AAAATAATTA CTTCTGCCCT GAGATAAGCA GATTAAGTTT GTTCATTCTC	4062
TGCTTTATTC TCTCCATGTG GCAACATTCT GTCAGCCTCT TTCATAGTGT GCAAACATTT	4122
TATCATTCTA AATGGTGAAT CTCTGCCCTT GGACCCATTT ATTATTCACA GATGGGGAGA	4182
ACCTATCTGC ATGGACCCTC ACCATCCTCT GTGCAGCACA CACAGTGCAG GGAGCCAGTG	4242
GCGATGGCGA TGACTTTCTT CCCCTG	4268

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 657 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

-112-

Glu Val Asp Val Leu Asp Val Asn Val Arg Gly Pro Asp Gly Cys Thr
 1 5 10 15
 Pro Leu Met Leu Ala Ser Leu Arg Gly Gly Ser Ser Asp Leu Ser Asp
 20 25 30
 Glu Asp Glu Asp Ala Glu Asp Ser Ser Ala Asn Ile Ile Thr Asp Leu
 35 40 45
 Val Tyr Gln Gly Ala Ser Leu Gln Ala Gln Thr Asp Arg Thr Gly Glu
 50 55 60
 Met Ala Leu His Leu Ala Ala Arg Tyr Ser Arg Ala Asp Ala Ala Lys
 65 70 75 80
 Arg Leu Leu Asp Ala Gly Ala Asp Ala Asn Ala Gln Asp Asn Met Gly
 85 90 95
 Arg Cys Pro Leu His Ala Ala Val Ala Ala Asp Ala Gln Gly Val Phe
 100 105 110
 Gln Ile Leu Ile Arg Asn Arg Val Thr Asp Leu Asp Ala Arg Met Asn
 115 120 125
 Asp Gly Thr Thr Pro Leu Ile Leu Ala Ala Arg Leu Ala Val Glu Gly
 130 135 140
 Met Val Ala Glu Leu Ile Asn Cys Gln Ala Asp Val Asn Ala Val Asp
 145 150 155 160
 Asp His Gly Lys Ser Ala Leu His Trp Ala Ala Ala Val Asn Asn Val
 165 170 175
 Glu Ala Thr Leu Leu Leu Lys Asn Gly Ala Asn Arg Asp Met Gln
 180 185 190
 Asp Asn Lys Glu Glu Thr Pro Leu Phe Leu Ala Ala Arg Glu Gly Ser
 195 200 205
 Tyr Glu Ala Ala Lys Ile Leu Leu Asp His Phe Ala Asn Arg Asp Ile
 210 215 220
 Thr Asp His Met Asp Arg Leu Pro Arg Asp Val Ala Arg Asp Arg Met
 225 230 235 240
 His His Asp Ile Val Arg Leu Leu Asp Glu Tyr Asn Val Thr Pro Ser
 245 250 255
 Pro Pro Gly Thr Val Leu Thr Ser Ala Leu Ser Pro Val Ile Cys Gly
 260 265 270
 Pro Asn Arg Ser Phe Leu Ser Leu Lys His Thr Pro Met Gly Lys Lys
 275 280 285
 Ser Arg Arg Pro Ser Ala Lys Ser Thr Met Pro Thr Ser Leu Pro Asn
 290 295 300
 Leu Ala Lys Glu Ala Lys Asp Ala Lys Gly Ser Arg Arg Lys Lys Ser
 305 310 315 320
 Leu Ser Glu Lys Val Gln Leu Ser Glu Ser Ser Val Thr Leu Ser Pro
 325 330 335
 Val Asp Ser Leu Glu Ser Pro His Thr Tyr Val Ser Asp Thr Thr Ser
 340 345 350
 Ser Pro Met Ile Thr Ser Pro Gly Ile Leu Gln Ala Ser Pro Asn Pro

-113-

355 360 365
 Met Leu Ala Thr Ala Ala Pro Pro Ala Pro Val His Ala Gln His Ala
 370 375 380
 Leu Ser Phe Ser Asn Leu His Glu Met Gln Pro Leu Ala His Gly Ala
 385 390 395 400
 Ser Thr Val Leu Pro Ser Val Ser Gln Leu Leu Ser His His His Ile
 405 410 415
 Val Ser Pro Gly Ser Gly Ser Ala Gly Ser Leu Ser Arg Leu His Pro
 420 425 430
 Val Pro Val Pro Ala Asp Trp Met Asn Arg Met Glu Val Asn Glu Thr
 435 440 445
 Gln Tyr Asn Glu Met Phe Gly Met Val Leu Ala Pro Ala Glu Gly Thr
 450 455 460
 His Pro Gly Ile Ala Pro Gln Ser Arg Pro Pro Glu Gly Lys His Ile
 465 470 475 480
 Thr Thr Pro Arg Glu Pro Leu Pro Pro Ile Val Thr Phe Gln Leu Ile
 485 490 495
 Pro Lys Gly Ser Ile Ala Gln Pro Ala Gly Ala Pro Gln Pro Gln Ser
 500 505 510
 Thr Cys Pro Pro Ala Val Ala Gly Pro Leu Pro Thr Met Tyr Gln Ile
 515 520 525
 Pro Glu Met Ala Arg Leu Pro Ser Val Ala Phe Pro Thr Ala Met Met
 530 535 540
 Pro Gln Gln Asp Gly Gln Val Ala Gln Thr Ile Leu Pro Ala Tyr His
 545 550 555 560
 Pro Phe Pro Ala Ser Val Gly Lys Tyr Pro Thr Pro Pro Ser Gln His
 565 570 575
 Ser Tyr Ala Ser Ser Asn Ala Ala Glu Arg Thr Pro Ser His Ser Gly
 580 585 590
 His Leu Gln Gly Glu His Pro Tyr Leu Thr Pro Ser Pro Glu Ser Pro
 595 600 605
 Asp Gln Trp Ser Ser Ser Ser Pro His Ser Ala Ser Asp Trp Ser Asp
 610 615 620
 Val Thr Thr Ser Pro Thr Pro Gly Gly Ala Gly Gly Gly Gln Arg Gly
 625 630 635 640
 Pro Gly Thr His Met Ser Glu Pro Pro His Asn Asn Met Gln Val Tyr
 645 650 655
 Ala

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

-114-

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Asp Ile Asp Glu Cys Asp Gln Gly Ser Pro Cys Glu His Asn Gly
 1 5 10 15
 Ile Cys Val Asn Thr Pro Gly Ser Tyr Arg Cys Asn Cys Ser Gln Gly
 20 25 30
 Phe Thr Gly Pro Arg Cys Glu Thr Asn Ile Asn Glu Cys Glu Ser His
 35 40 45
 Pro Cys Gln Asn Glu Gly Ser Cys Leu Asp Asp Pro Gly Thr Phe Arg
 50 55 60
 Cys Val Cys Met Pro Gly Phe Thr Gly Thr Gln Cys Glu
 65 70 75

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 78 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Asn Asp Val Asp Glu Cys Ser Leu Gly Ala Asn Pro Cys Glu His Gly
 1 5 10 15
 Gly Arg Cys Thr Asn Thr Leu Gly Ser Phe Gln Cys Asn Cys Pro Gln
 20 25 30
 Gly Tyr Ala Gly Pro Arg Cys Glu Ile Asp Val Asn Glu Cys Leu Ser
 35 40 45
 Asn Pro Cys Gln Asn Asp Ser Thr Cys Leu Asp Gln Ile Gly Glu Phe
 50 55 60
 Gln Cys Ile Cys Met Pro Gly Tyr Glu Gly Leu Tyr Cys Glu
 65 70 75

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 654 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Thr Pro Pro Gln Gly Glu Ile Glu Ala Asp Cys Met Asp Val Asn Val

-115-

1	5	10	15
Arg Gly Pro Asp Gly Phe Thr Pro Leu Met Ile Ala Ser Cys Ser Gly	20	25	30
Gly Gly Leu Glu Thr Gly Asn Ser Glu Glu Glu Glu Asp Ala Ser Ala	35	40	45
Asn Met Ile Ser Asp Phe Ile Gly Gln Gly Ala Gln Leu His Asn Gln	50	55	60
Thr Asp Arg Thr Gly Glu Thr Ala Leu His Leu Ala Ala Arg Tyr Ala	65	70	75
Arg Ala Asp Ala Ala Lys Arg Leu Leu Glu Ser Ser Ala Asp Ala Asn	85	90	95
Val Gln Asp Asn Met Gly Arg Thr Pro Leu His Ala Ala Val Ala Ala	100	105	110
Asp Ala Gln Gly Val Phe Gln Ile Leu Ile Arg Asn Arg Ala Thr Asp	115	120	125
Leu Asp Ala Arg Met Phe Asp Gly Thr Thr Pro Leu Ile Leu Ala Ala	130	135	140
Arg Leu Ala Val Glu Gly Met Val Glu Glu Leu Ile Asn Ala His Ala	145	150	155
Asp Val Asn Ala Val Asp Glu Phe Gly Lys Ser Ala Leu His Trp Ala	165	170	175
Ala Ala Val Asn Asn Val Asp Ala Ala Ala Val Leu Leu Lys Asn Ser	180	185	190
Ala Asn Lys Asp Met Gln Asn Asn Lys Glu Glu Thr Ser Leu Phe Leu	195	200	205
Ala Ala Arg Glu Gly Ser Tyr Glu Thr Ala Lys Val Leu Leu Asp His	210	215	220
Tyr Ala Asn Arg Asp Ile Thr Asp His Met Asp Arg Leu Pro Arg Asp	225	230	235
Ile Ala Gln Glu Arg Met His His Asp Ile Val His Leu Leu Asp Glu	245	250	255
Tyr Asn Leu Val Lys Ser Pro Thr Leu His Asn Gly Pro Leu Gly Ala	260	265	270
Thr Thr Leu Ser Pro Pro Ile Cys Ser Pro Asn Gly Tyr Met Gly Asn	275	280	285
Met Lys Pro Ser Val Gln Ser Lys Lys Ala Arg Lys Pro Ser Ile Lys	290	295	300
Gly Asn Gly Cys Lys Glu Ala Lys Glu Leu Lys Ala Arg Arg Lys Lys	305	310	315
Ser Gln Asp Gly Lys Thr Thr Leu Leu Asp Ser Gly Ser Ser Gly Val	325	330	335
Leu Ser Pro Val Asp Ser Leu Glu Ser Thr His Gly Tyr Leu Ser Asp	340	345	350
Val Ser Ser Pro Pro Leu Met Thr Ser Pro Phe Gln Gln Ser Pro Ser	355	360	365

-116-

Met Pro Leu Asn His Leu Thr Ser Met Pro Glu Ser Gln Leu Gly Met
 370 375 380

Asn His Ile Asn Met Ala Thr Lys Gln Glu Met Ala Ala Gly Ser Asn
 385 390 395 400

Arg Met Ala Phe Asp Ala Met Val Pro Arg Leu Thr His Leu Asn Ala
 405 410 415

Ser Ser Pro Asn Thr Ile Met Ser Asn Gly Ser Met His Phe Thr Val
 420 425 430

Gly Gly Ala Pro Thr Met Asn Ser Gln Cys Asp Trp Leu Ala Arg Leu
 435 440 445

Gln Asn Gly Met Val Gln Asn Gln Tyr Asp Pro Ile Arg Asn Gly Ile
 450 455 460

Gln Gln Gly Asn Ala Gln Gln Ala Gln Ala Leu Gln His Gly Leu Met
 465 470 475 480

Thr Ser Leu His Asn Gly Leu Pro Ala Thr Thr Leu Ser Gln Met Met
 485 490 495

Thr Tyr Gln Ala Met Pro Asn Thr Arg Leu Ala Asn Gln Pro His Leu
 500 505 510

Met Gln Ala Gln Gln Met Gln Gln Gln Asn Leu Gln Leu His Gln
 515 520 525

Ser Met Gln Gln Gln His His Asn Ser Ser Thr Thr Ser Thr His Ile
 530 535 540

Asn Ser Pro Phe Cys Ser Ser Asp Ile Ser Gln Thr Asp Leu Gln Gln
 545 550 555 560

Met Ser Ser Asn Asn Ile His Ser Val Met Pro Gln Asp Thr Gln Ile
 565 570 575

Phe Ala Ala Ser Leu Pro Ser Asn Leu Thr Gln Ser Met Thr Thr Ala
 580 585 590

Gln Phe Leu Thr Pro Pro Ser Gln His Ser Tyr Ser Ser Pro Met Asp
 595 600 605

Asn Thr Pro Ser His Gln Leu Gln Val Pro Asp His Pro Phe Leu Thr
 610 615 620

Pro Ser Pro Glu Ser Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser
 625 630 635 640

Asn Met Ser Asp Trp Ser Glu Gly Ile Ser Ser Pro Pro Thr
 645 650

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 666 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

-117-

Thr	Pro	Pro	Gln	Gly	Glu	Val	Asp	Ala	Asp	Cys	Met	Asp	Val	Asn	Val	
1				5					10					15		
Arg	Gly	Pro	Asp	Gly	Phe	Thr	Pro	Leu	Met	Ile	Ala	Ser	Cys	Ser	Gly	
			20					25					30			
Gly	Gly	Leu	Glu	Thr	Gly	Asn	Ser	Glu	Glu	Glu	Glu	Asp	Ala	Pro	Ala	
		35					40					45				
Val	Ile	Ser	Asp	Phe	Ile	Tyr	Gln	Gly	Ala	Ser	Leu	His	Asn	Gln	Thr	
	50					55					60					
Asp	Arg	Thr	Gly	Glu	Thr	Ala	Leu	His	Leu	Ala	Ala	Arg	Tyr	Ser	Arg	
65					70				75						80	
Ser	Asp	Ala	Ala	Lys	Arg	Leu	Leu	Glu	Ala	Ser	Ala	Asp	Ala	Asn	Ile	
				85					90					95		
Gln	Asp	Asn	Met	Gly	Arg	Thr	Pro	Leu	His	Ala	Ala	Val	Ser	Ala	Asp	
			100					105					110			
Ala	Gln	Gly	Val	Phe	Gln	Ile	Leu	Leu	Arg	Asn	Arg	Ala	Thr	Asp	Leu	
		115					120					125				
Asp	Ala	Arg	Met	His	Asp	Gly	Thr	Thr	Pro	Leu	Ile	Leu	Ala	Ala	Arg	
	130					135					140					
Leu	Ala	Val	Glu	Gly	Met	Leu	Glu	Asp	Leu	Ile	Asn	Ser	His	Ala	Asp	
145					150					155					160	
Val	Asn	Ala	Val	Asp	Asp	Leu	Gly	Lys	Ser	Ala	Leu	His	Trp	Ala	Ala	
				165					170					175		
Ala	Val	Asn	Asn	Val	Asp	Ala	Ala	Val	Val	Leu	Leu	Lys	Asn	Gly	Ala	
			180					185					190			
Asn	Lys	Asp	Met	Gln	Asn	Asn	Lys	Glu	Glu	Thr	Pro	Leu	Phe	Leu	Ala	
		195					200					205				
Ala	Arg	Glu	Gly	Ser	Tyr	Glu	Thr	Ala	Lys	Val	Leu	Leu	Asp	His	Phe	
	210					215					220					
Ala	Asn	Arg	Asp	Ile	Thr	Asp	His	Met	Asp	Arg	Leu	Pro	Arg	Asp	Ile	
225					230					235					240	
Ala	Gln	Glu	Arg	Met	His	His	Asp	Ile	Val	Arg	Leu	Leu	Asp	Glu	Tyr	
				245					250					255		
Asn	Leu	Val	Arg	Ser	Pro	Gln	Leu	His	Gly	Thr	Ala	Leu	Gly	Gly	Thr	
			260					265					270			
Pro	Thr	Leu	Ser	Pro	Thr	Leu	Cys	Ser	Pro	Asn	Gly	Tyr	Leu	Gly	Asn	
		275					280					285				
Leu	Lys	Ser	Ala	Thr	Gln	Gly	Lys	Lys	Ala	Arg	Lys	Pro	Ser	Thr	Lys	
	290					295					300					
Gly	Leu	Ala	Cys	Ser	Ser	Lys	Glu	Ala	Lys	Asp	Leu	Lys	Ala	Arg	Arg	
305					310					315					320	
Lys	Lys	Ser	Gln	Asp	Gly	Lys	Gly	Cys	Leu	Leu	Asp	Ser	Ser	Ser	Met	
				325					330					335		
Leu	Ser	Pro	Val	Asp	Ser	Leu	Glu	Ser	Pro	His	Gly	Tyr	Leu	Ser	Asp	
			340					345					350			
Val	Ala	Ser	Pro	Pro	Leu	Pro	Ser	Pro	Phe	Gln	Gln	Ser	Pro	Ser	Met	

-118-

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355                      360                      365
Pro Leu Ser His Leu Pro Gly Met Pro Asp Thr His Leu Gly Ile Ser
370                      375                      380
His Leu Asn Val Ala Ala Lys Pro Glu Met Ala Ala Leu Ala Gly Gly
385                      390                      395                      400
Ser Arg Leu Ala Phe Glu Pro Pro Pro Pro Arg Leu Ser His Leu Pro
405                      410                      415
Val Ala Ser Ser Ala Ser Thr Val Leu Ser Thr Asn Gly Thr Gly Ala
420                      425                      430
Met Asn Phe Thr Val Gly Ala Pro Ala Ser Leu Asn Gly Gln Cys Glu
435                      440                      445
Trp Leu Pro Arg Leu Gln Asn Gly Met Val Pro Ser Gln Tyr Asn Pro
450                      455                      460
Leu Arg Pro Gly Val Thr Pro Gly Thr Leu Ser Thr Gln Ala Ala Gly
465                      470                      475                      480
Leu Gln His Gly Met Met Ser Pro Ile His Ser Ser Leu Ser Thr Asn
485                      490                      495
Thr Leu Ser Pro Ile Ile Tyr Gln Gly Leu Pro Asn Thr Arg Leu Ala
500                      505                      510
Thr Gln Pro His Leu Val Gln Thr Gln Gln Val Gln Pro Gln Asn Leu
515                      520                      525
Gln Ile Gln Pro Gln Asn Leu Gln Pro Pro Ser Gln Pro His Leu Ser
530                      535                      540
Val Ser Ser Ala Ala Asn Gly His Leu Gly Arg Ser Phe Leu Ser Gly
545                      550                      555                      560
Glu Pro Ser Gln Ala Asp Val Gln Pro Leu Gly Pro Ser Ser Leu Pro
565                      570                      575
Val His Thr Ile Leu Pro Gln Glu Ser Gln Ala Leu Pro Thr Ser Leu
580                      585                      590
Pro Ser Ser Met Val Pro Pro Met Thr Thr Thr Gln Phe Leu Thr Pro
595                      600                      605
Pro Ser Gln His Ser Tyr Ser Ser Ser Pro Val Asp Asn Thr Pro Ser
610                      615                      620
His Gln Leu Gln Val Pro Glu His Pro Phe Leu Thr Pro Ser Pro Glu
625                      630                      635                      640
Ser Pro Asp Gln Trp Ser Ser Ser Ser Arg His Ser Asn Ile Ser Asp
645                      650                      655
Trp Ser Glu Gly Ile Ser Ser Pro Pro Thr
660                      665

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(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 681 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

-119-

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Thr	Pro	Pro	Gln	Gly	Glu	Val	Asp	Ala	Asp	Cys	Met	Asp	Val	Asn	Val	1	5	10	15
Arg	Gly	Pro	Asp	Gly	Phe	Thr	Pro	Leu	Met	Ile	Ala	Ser	Cys	Ser	Gly	20	25	30	
Gly	Gly	Leu	Glu	Thr	Gly	Asn	Ser	Glu	Glu	Glu	Glu	Asp	Ala	Pro	Ala	35	40	45	
Val	Ile	Ser	Asp	Phe	Ile	Tyr	Gln	Gly	Ala	Ser	Leu	His	Asn	Gln	Thr	50	55	60	
Asp	Arg	Thr	Gly	Glu	Thr	Ala	Leu	His	Leu	Ala	Ala	Arg	Tyr	Ser	Arg	65	70	75	80
Ser	Asp	Ala	Ala	Lys	Arg	Leu	Leu	Glu	Ala	Ser	Ala	Asp	Ala	Asn	Ile	85	90	95	
Gln	Asp	Asn	Met	Gly	Arg	Thr	Pro	Leu	His	Ala	Ala	Val	Ser	Ala	Asp	100	105	110	
Ala	Gln	Gly	Val	Phe	Gln	Ile	Leu	Ile	Arg	Asn	Arg	Ala	Thr	Asp	Leu	115	120	125	
Asp	Ala	Arg	Met	His	Asp	Gly	Thr	Thr	Pro	Leu	Ile	Leu	Ala	Ala	Arg	130	135	140	
Leu	Ala	Val	Glu	Gly	Met	Leu	Glu	Asp	Leu	Ile	Asn	Ser	His	Ala	Asp	145	150	155	160
Val	Asn	Ala	Val	Asp	Asp	Leu	Gly	Lys	Ser	Ala	Leu	His	Trp	Ala	Ala	165	170	175	
Ala	Val	Asn	Asn	Val	Asp	Ala	Ala	Val	Val	Leu	Leu	Lys	Asn	Gly	Ala	180	185	190	
Asn	Lys	Asp	Met	Gln	Asn	Asn	Arg	Glu	Glu	Thr	Pro	Leu	Phe	Leu	Ala	195	200	205	
Ala	Arg	Glu	Gly	Ser	Tyr	Glu	Thr	Ala	Lys	Val	Leu	Leu	Asp	His	Phe	210	215	220	
Ala	Asn	Arg	Asp	Ile	Thr	Asp	His	Met	Asp	Arg	Leu	Pro	Arg	Asp	Ile	225	230	235	240
Ala	Gln	Glu	Arg	Met	His	His	Asp	Ile	Val	Arg	Leu	Leu	Asp	Glu	Tyr	245	250	255	
Asn	Leu	Val	Arg	Ser	Pro	Gln	Leu	His	Gly	Ala	Pro	Leu	Gly	Gly	Thr	260	265	270	
Pro	Thr	Leu	Ser	Pro	Pro	Leu	Cys	Ser	Pro	Asn	Gly	Tyr	Leu	Gly	Ser	275	280	285	
Leu	Lys	Pro	Gly	Val	Gln	Gly	Lys	Lys	Val	Arg	Lys	Pro	Ser	Ser	Lys	290	295	300	
Gly	Leu	Ala	Cys	Gly	Ser	Lys	Glu	Ala	Lys	Asp	Leu	Lys	Ala	Arg	Arg	305	310	315	320
Lys	Lys	Ser	Gln	Asp	Gly	Lys	Gly	Cys	Leu	Leu	Asp	Ser	Ser	Gly	Met				

325

330

335

ENSDOCID: WQ 9407474A1_1

-121-

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2471 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Pro Ala Leu Arg Pro Ala Leu Leu Trp Ala Leu Leu Ala Leu Trp
 1 5 10 15
 Leu Cys Cys Ala Ala Pro Ala His Ala Leu Gln Cys Arg Asp Gly Tyr
 20 25 30
 Glu Pro Cys Val Asn Glu Gly Met Cys Val Thr Tyr His Asn Gly Thr
 35 40 45
 Gly Tyr Cys Lys Cys Pro Glu Gly Phe Leu Gly Glu Tyr Cys Gln His
 50 55 60
 Arg Asp Pro Cys Glu Lys Asn Arg Cys Gln Asn Gly Gly Thr Cys Val
 65 70 75 80
 Ala Gln Ala Met Leu Gly Lys Ala Thr Cys Arg Cys Ala Ser Gly Phe
 85 90 95
 Thr Gly Glu Asp Cys Gln Tyr Ser Thr Ser His Pro Cys Phe Val Ser
 100 105 110
 Arg Pro Cys Leu Asn Gly Gly Thr Cys His Met Leu Ser Arg Asp Thr
 115 120 125
 Tyr Glu Cys Thr Cys Gln Val Gly Phe Thr Gly Lys Glu Cys Gln Trp
 130 135 140
 Thr Asp Ala Cys Leu Ser His Pro Cys Ala Asn Gly Ser Thr Cys Thr
 145 150 155 160
 Thr Val Ala Asn Gln Phe Ser Cys Lys Cys Leu Thr Gly Phe Thr Gly
 165 170 175
 Gln Lys Cys Glu Thr Asp Val Asn Glu Cys Asp Ile Pro Gly His Cys
 180 185 190
 Gln His Gly Gly Thr Cys Leu Asn Leu Pro Gly Ser Tyr Gln Cys Gln
 195 200 205
 Cys Pro Gln Gly Phe Thr Gly Gln Tyr Cys Asp Ser Leu Tyr Val Pro
 210 215 220
 Cys Ala Pro Ser Pro Cys Val Asn Gly Gly Thr Cys Arg Gln Thr Gly
 225 230 235 240
 Asp Phe Thr Phe Glu Cys Asn Cys Leu Pro Gly Phe Glu Gly Ser Thr
 245 250 255
 Cys Glu Arg Asn Ile Asp Asp Cys Pro Asn His Arg Cys Gln Asn Gly
 260 265 270
 Gly Val Cys Val Asp Gly Val Asn Thr Tyr Asn Cys Arg Cys Pro Pro
 275 280 285

-122-

Gln Trp Thr Gly Gln Phe Cys Thr Glu Asp Val Asp Glu Cys Leu Leu
 290 295 300
 Gln Pro Asn Ala Cys Gln Asn Gly Gly Thr Cys Ala Asn Arg Asn Gly
 305 310 315 320
 Gly Tyr Gly Cys Val Cys Val Asn Gly Trp Ser Gly Asp Asp Cys Ser
 325 330 335
 Glu Asn Ile Asp Asp Cys Ala Phe Ala Ser Cys Thr Pro Gly Ser Thr
 340 345 350
 Cys Ile Asp Arg Val Ala Ser Phe Ser Cys Met Cys Pro Glu Gly Lys
 355 360 365
 Ala Gly Leu Leu Cys His Leu Asp Asp Ala Cys Ile Ser Asn Pro Cys
 370 375 380
 His Lys Gly Ala Leu Cys Asp Thr Asn Pro Leu Asn Gly Gln Tyr Ile
 385 390 395 400
 Cys Thr Cys Pro Gln Gly Tyr Lys Gly Ala Asp Cys Thr Glu Asp Val
 405 410 415
 Asp Glu Cys Ala Met Ala Asn Ser Asn Pro Cys Glu His Ala Gly Lys
 420 425 430
 Cys Val Asn Thr Asp Gly Ala Phe His Cys Glu Cys Leu Lys Gly Tyr
 435 440 445
 Ala Gly Pro Arg Cys Glu Met Asp Ile Asn Glu Cys His Ser Asp Pro
 450 455 460
 Cys Gln Asn Asp Ala Thr Cys Leu Asp Lys Ile Gly Gly Phe Thr Cys
 465 470 475 480
 Leu Cys Met Pro Gly Phe Lys Gly Val His Cys Glu Leu Glu Ile Asn
 485 490 495
 Glu Cys Gln Ser Asn Pro Cys Val Asn Asn Gly Gln Cys Val Asp Lys
 500 505 510
 Val Asn Arg Phe Gln Cys Leu Cys Pro Pro Gly Phe Thr Gly Pro Val
 515 520 525
 Cys Gln Ile Asp Ile Asp Asp Cys Ser Ser Thr Pro Cys Leu Asn Gly
 530 535 540
 Ala Lys Cys Ile Asp His Pro Asn Gly Tyr Glu Cys Gln Cys Ala Thr
 545 550 555 560
 Gly Phe Thr Gly Val Leu Cys Glu Glu Asn Ile Asp Asn Cys Asp Pro
 565 570 575
 Asp Pro Cys His His Gly Gln Cys Gln Asp Gly Ile Asp Ser Tyr Thr
 580 585 590
 Cys Ile Cys Asn Pro Gly Tyr Met Gly Ala Ile Cys Ser Asp Gln Ile
 595 600 605
 Asp Glu Cys Tyr Ser Ser Pro Cys Leu Asn Asp Gly Arg Cys Ile Asp
 610 615 620
 Leu Val Asn Gly Tyr Gln Cys Asn Cys Gln Pro Gly Thr Ser Gly Val
 625 630 635 640
 Asn Cys Glu Ile Asn Phe Asp Asp Cys Ala Ser Asn Pro Cys Ile His

-123-

645	650	655
Gly Ile Cys Met Asp Gly Ile Asn Arg Tyr Ser Cys Val Cys Ser Pro		
660	665	670
Gly Phe Thr Gly Gln Arg Cys Asn Ile Asp Ile Asp Glu Cys Ala Ser		
675	680	685
Asn Pro Cys Arg Lys Gly Ala Thr Cys Ile Asn Gly Val Asn Gly Phe		
690	695	700
Arg Cys Ile Cys Pro Glu Gly Pro His His Pro Ser Cys Tyr Ser Gln		
705	710	715
Val Asn Glu Cys Leu Ser Asn Pro Cys Ile His Gly Asn Cys Thr Gly		
725	730	735
Gly Leu Ser Gly Tyr Lys Cys Leu Cys Asp Ala Gly Trp Val Gly Ile		
740	745	750
Asn Cys Glu Val Asp Lys Asn Glu Cys Leu Ser Asn Pro Cys Gln Asn		
755	760	765
Gly Gly Thr Cys Asp Asn Leu Val Asn Gly Tyr Arg Cys Thr Cys Lys		
770	775	780
Lys Gly Phe Lys Gly Tyr Asn Cys Gln Val Asn Ile Asp Glu Cys Ala		
785	790	795
Ser Asn Pro Cys Leu Asn Gln Gly Thr Cys Phe Asp Asp Ile Ser Gly		
805	810	815
Tyr Thr Cys His Cys Val Leu Pro Tyr Thr Gly Lys Asn Cys Gln Thr		
820	825	830
Val Leu Ala Pro Cys Ser Pro Asn Pro Cys Glu Asn Ala Ala Val Cys		
835	840	845
Lys Glu Ser Pro Asn Phe Glu Ser Tyr Thr Cys Leu Cys Ala Pro Gly		
850	855	860
Trp Gln Gly Gln Arg Cys Thr Ile Asp Ile Asp Glu Cys Ile Ser Lys		
865	870	875
Pro Cys Met Asn His Gly Leu Cys His Asn Thr Gln Gly Ser Tyr Met		
885	890	895
Cys Glu Cys Pro Pro Gly Phe Ser Gly Met Asp Cys Glu Glu Asp Ile		
900	905	910
Asp Asp Cys Leu Ala Asn Pro Cys Gln Asn Gly Gly Ser Cys Met Asp		
915	920	925
Gly Val Asn Thr Phe Ser Cys Leu Cys Leu Pro Gly Phe Thr Gly Asp		
930	935	940
Lys Cys Gln Thr Asp Met Asn Glu Cys Leu Ser Glu Pro Cys Lys Asn		
945	950	955
Gly Gly Thr Cys Ser Asp Tyr Val Asn Ser Tyr Thr Cys Lys Cys Gln		
965	970	975
Ala Gly Phe Asp Gly Val His Cys Glu Asn Asn Ile Asn Glu Cys Thr		
980	985	990
Glu Ser Ser Cys Phe Asn Gly Gly Thr Cys Val Asp Gly Ile Asn Ser		
995	1000	1005

-124-

Phe Ser Cys Leu Cys Pro Val Gly Phe Thr Gly Ser Phe Cys Leu His
 1010 1015 1020
 Glu Ile Asn Glu Cys Ser Ser His Pro Cys Leu Asn Glu Gly Thr Cys
 1025 1030 1035 1040
 Val Asp Gly Leu Gly Thr Tyr Arg Cys Ser Cys Pro Leu Gly Tyr Thr
 1045 1050 1055
 Gly Lys Asn Cys Gln Thr Leu Val Asn Leu Cys Ser Arg Ser Pro Cys
 1060 1065 1070
 Lys Asn Lys Gly Thr Cys Val Gln Lys Lys Ala Glu Ser Gln Cys Leu
 1075 1080 1085
 Cys Pro Ser Gly Trp Ala Gly Ala Tyr Cys Asp Val Pro Asn Val Ser
 1090 1095 1100
 Cys Asp Ile Ala Ala Ser Arg Arg Gly Val Leu Val Glu His Leu Cys
 1105 1110 1115 1120
 Gln His Ser Gly Val Cys Ile Asn Ala Gly Asn Thr His Tyr Cys Gln
 1125 1130 1135
 Cys Pro Leu Gly Tyr Thr Gly Ser Tyr Cys Glu Glu Gln Leu Asp Glu
 1140 1145 1150
 Cys Ala Ser Asn Pro Cys Gln His Gly Ala Thr Cys Ser Asp Phe Ile
 1155 1160 1165
 Gly Gly Tyr Arg Cys Glu Cys Val Pro Gly Tyr Gln Gly Val Asn Cys
 1170 1175 1180
 Glu Tyr Glu Val Asp Glu Cys Gln Asn Gln Pro Cys Gln Asn Gly Gly
 1185 1190 1195 1200
 Thr Cys Ile Asp Leu Val Asn His Phe Lys Cys Ser Cys Pro Pro Gly
 1205 1210 1215
 Thr Arg Gly Leu Leu Cys Glu Glu Asn Ile Asp Asp Cys Ala Arg Gly
 1220 1225 1230
 Pro His Cys Leu Asn Gly Gly Gln Cys Met Asp Arg Ile Gly Gly Tyr
 1235 1240 1245
 Ser Cys Arg Cys Leu Pro Gly Phe Ala Gly Glu Arg Cys Glu Gly Asp
 1250 1255 1260
 Ile Asn Glu Cys Leu Ser Asn Pro Cys Ser Ser Glu Gly Ser Leu Asp
 1265 1270 1275 1280
 Cys Ile Gln Leu Thr Asn Asp Tyr Leu Cys Val Cys Arg Ser Ala Phe
 1285 1290 1295
 Thr Gly Arg His Cys Glu Thr Phe Val Asp Val Cys Pro Gln Met Pro
 1300 1305 1310
 Cys Leu Asn Gly Gly Thr Cys Ala Val Ala Ser Asn Met Pro Asp Gly
 1315 1320 1325
 Phe Ile Cys Arg Cys Pro Pro Gly Phe Ser Gly Ala Arg Cys Gln Ser
 1330 1335 1340
 Ser Cys Gly Gln Val Lys Cys Arg Lys Gly Glu Gln Cys Val His Thr
 1345 1350 1355 1360
 Ala Ser Gly Pro Arg Cys Phe Cys Pro Ser Pro Arg Asp Cys Glu Ser

-125-

1365	1370	1375
Gly Cys Ala Ser Ser Pro Cys Gln His Gly Gly Ser Cys His Pro Gln 1380 1385 1390		
Arg Gln Pro Pro Tyr Tyr Ser Cys Gln Cys Ala Pro Pro Phe Ser Gly 1395 1400 1405		
Ser Arg Cys Glu Leu Tyr Thr Ala Pro Pro Ser Thr Pro Pro Ala Thr 1410 1415 1420		
Cys Leu Ser Gln Tyr Cys Ala Asp Lys Ala Arg Asp Gly Val Cys Asp 1425 1430 1435 1440		
Glu Ala Cys Asn Ser His Ala Cys Gln Trp Asp Gly Gly Asp Cys Ser 1445 1450 1455		
Leu Thr Met Glu Asn Pro Trp Ala Asn Cys Ser Ser Pro Leu Pro Cys 1460 1465 1470		
Trp Asp Tyr Ile Asn Asn Gln Cys Asp Glu Leu Cys Asn Thr Val Glu 1475 1480 1485		
Cys Leu Phe Asp Asn Phe Glu Cys Gln Gly Asn Ser Lys Thr Cys Lys 1490 1495 1500		
Tyr Asp Lys Tyr Cys Ala Asp His Phe Lys Asp Asn His Cys Asn Gln 1505 1510 1515 1520		
Gly Cys Asn Ser Glu Glu Cys Gly Trp Asp Gly Leu Asp Cys Ala Ala 1525 1530 1535		
Asp Gln Pro Glu Asn Leu Ala Glu Gly Thr Leu Val Ile Val Val Leu 1540 1545 1550		
Met Pro Pro Glu Gln Leu Leu Gln Asp Ala Arg Ser Phe Leu Arg Ala 1555 1560 1565		
Leu Gly Thr Leu Leu His Thr Asn Leu Arg Ile Lys Arg Asp Ser Gln 1570 1575 1580		
Gly Glu Leu Met Val Tyr Pro Tyr Tyr Gly Glu Lys Ser Ala Ala Met 1585 1590 1595 1600		
Lys Lys Gln Arg Met Thr Arg Arg Ser Leu Pro Gly Glu Gln Glu Gln 1605 1610 1615		
Glu Val Ala Gly Ser Lys Val Phe Leu Glu Ile Asp Asn Arg Gln Cys 1620 1625 1630		
Val Gln Asp Ser Asp His Cys Phe Lys Asn Thr Asp Ala Ala Ala Ala 1635 1640 1645		
Leu Leu Ala Ser His Ala Ile Gln Gly Thr Leu Ser Tyr Pro Leu Val 1650 1655 1660		
Ser Val Val Ser Glu Ser Leu Thr Pro Glu Arg Thr Gln Leu Leu Tyr 1665 1670 1675 1680		
Leu Leu Ala Val Ala Val Val Ile Ile Leu Phe Ile Ile Leu Leu Gly 1685 1690 1695		
Val Ile Met Ala Lys Arg Lys Arg Lys His Gly Ser Leu Trp Leu Pro 1700 1705 1710		
Glu Gly Phe Thr Leu Arg Arg Asp Ala Ser Asn His Lys Arg Arg Glu 1715 1720 1725		

-126-

Pro Val Gly Gln Asp Ala Val Gly Leu Lys Asn Leu Ser Val Gln Val
 1730 1735 1740
 Ser Glu Ala Asn Leu Ile Gly Thr Gly Thr Ser Glu His Trp Val Asp
 1745 1750 1755 1760
 Asp Glu Gly Pro Gln Pro Lys Lys Val Lys Ala Glu Asp Glu Ala Leu
 1765 1770 1775
 Leu Ser Glu Glu Asp Asp Pro Ile Asp Arg Arg Pro Trp Thr Gln Gln
 1780 1785 1790
 His Leu Glu Ala Ala Asp Ile Arg Arg Thr Pro Ser Leu Ala Leu Thr
 1795 1800 1805
 Pro Pro Gln Ala Glu Gln Glu Val Asp Val Leu Asp Val Asn Val Arg
 1810 1815 1820
 Gly Pro Asp Gly Cys Thr Pro Leu Met Leu Ala Ser Leu Arg Gly Gly
 1825 1830 1835 1840
 Ser Ser Asp Leu Ser Asp Glu Asp Glu Asp Ala Glu Asp Ser Ser Ala
 1845 1850 1855
 Asn Ile Ile Thr Asp Leu Val Tyr Gln Gly Ala Ser Leu Gln Ala Gln
 1860 1865 1870
 Thr Asp Arg Thr Gly Glu Met Ala Leu His Leu Ala Ala Arg Tyr Ser
 1875 1880 1885
 Arg Ala Asp Ala Ala Lys Arg Leu Leu Asp Ala Gly Ala Asp Ala Asn
 1890 1895 1900
 Ala Gln Asp Asn Met Gly Arg Cys Pro Leu His Ala Ala Val Ala Ala
 1905 1910 1915 1920
 Asp Ala Gln Gly Val Phe Gln Ile Leu Ile Arg Asn Arg Val Thr Asp
 1925 1930 1935
 Leu Asp Ala Arg Met Asn Asp Gly Thr Thr Pro Leu Ile Leu Ala Ala
 1940 1945 1950
 Arg Leu Ala Val Glu Gly Met Val Ala Glu Leu Ile Asn Cys Gln Ala
 1955 1960 1965
 Asp Val Asn Ala Val Asp Asp His Gly Lys Ser Ala Leu His Trp Ala
 1970 1975 1980
 Ala Ala Val Asn Asn Val Glu Ala Thr Leu Leu Leu Leu Lys Asn Gly
 1985 1990 1995 2000
 Ala Asn Arg Asp Met Gln Asp Asn Lys Glu Glu Thr Pro Leu Phe Leu
 2005 2010 2015
 Ala Ala Arg Glu Gly Ser Tyr Glu Ala Ala Lys Ile Leu Leu Asp His
 2020 2025 2030
 Phe Ala Asn Arg Asp Ile Thr Asp His Met Asp Arg Leu Pro Arg Asp
 2035 2040 2045
 Val Ala Arg Asp Arg Met His His Asp Ile Val Arg Leu Leu Asp Glu
 2050 2055 2060
 Tyr Asn Val Thr Pro Ser Pro Pro Gly Thr Val Leu Thr Ser Ala Leu
 2065 2070 2075 2080
 Ser Pro Val Ile Cys Gly Pro Asn Arg Ser Phe Leu Ser Leu Lys His

2085

2090

2095

Thr	Pro	Met	Gly	Lys	Lys	Ser	Arg	Pro	Ser	Ala	Lys	Ser	Thr	Met	2100	2105	2110	
Pro	Thr	Ser	Leu	Pro	Asn	Leu	Ala	Lys	Glu	Ala	Lys	Asp	Ala	Lys	Gly	2115	2120	2125
Ser	Arg	Arg	Lys	Lys	Ser	Leu	Ser	Glu	Lys	Val	Gln	Leu	Ser	Glu	Ser	2130	2135	2140
Ser	Val	Thr	Leu	Ser	Pro	Val	Asp	Ser	Leu	Glu	Ser	Pro	His	Thr	Tyr	2145	2150	2155
Val	Ser	Asp	Thr	Thr	Ser	Ser	Pro	Met	Ile	Thr	Ser	Pro	Gly	Ile	Leu	2165	2170	2175
Gln	Ala	Ser	Pro	Asn	Pro	Met	Leu	Ala	Thr	Ala	Ala	Pro	Pro	Ala	Pro	2180	2185	2190
Val	His	Ala	Gln	His	Ala	Leu	Ser	Phe	Ser	Asn	Leu	His	Glu	Met	Gln	2195	2200	2205
Pro	Leu	Ala	His	Gly	Ala	Ser	Thr	Val	Leu	Pro	Ser	Val	Ser	Gln	Leu	2210	2215	2220
Leu	Ser	His	His	His	Ile	Val	Ser	Pro	Gly	Ser	Gly	Ser	Ala	Gly	Ser	2225	2230	2235
Leu	Ser	Arg	Leu	His	Pro	Val	Pro	Val	Pro	Ala	Asp	Trp	Met	Asn	Arg	2245	2250	2255
Met	Glu	Val	Asn	Glu	Thr	Gln	Tyr	Asn	Glu	Met	Phe	Gly	Met	Val	Leu	2260	2265	2270
Ala	Pro	Ala	Glu	Gly	Thr	His	Pro	Gly	Ile	Ala	Pro	Gln	Ser	Arg	Pro	2275	2280	2285
Pro	Glu	Gly	Lys	His	Ile	Thr	Thr	Pro	Arg	Glu	Pro	Leu	Pro	Pro	Ile	2290	2295	2300
Val	Thr	Phe	Gln	Leu	Ile	Pro	Lys	Gly	Ser	Ile	Ala	Gln	Pro	Ala	Gly	2305	2310	2315
Ala	Pro	Gln	Pro	Gln	Ser	Thr	Cys	Pro	Pro	Ala	Val	Ala	Gly	Pro	Leu	2325	2330	2335
Pro	Thr	Met	Tyr	Gln	Ile	Pro	Glu	Met	Ala	Arg	Leu	Pro	Ser	Val	Ala	2340	2345	2350
Phe	Pro	Thr	Ala	Met	Met	Pro	Gln	Gln	Asp	Gly	Gln	Val	Ala	Gln	Thr	2355	2360	2365
Ile	Leu	Pro	Ala	Tyr	His	Pro	Phe	Pro	Ala	Ser	Val	Gly	Lys	Tyr	Pro	2370	2375	2380
Thr	Pro	Pro	Ser	Gln	His	Ser	Tyr	Ala	Ser	Ser	Asn	Ala	Ala	Glu	Arg	2385	2390	2395
Thr	Pro	Ser	His	Ser	Gly	His	Leu	Gln	Gly	Glu	His	Pro	Tyr	Leu	Thr	2405	2410	2415
Pro	Ser	Pro	Glu	Ser	Pro	Asp	Gln	Trp	Ser	Ser	Ser	Ser	Pro	His	Ser	2420	2425	2430
Ala	Ser	Asp	Trp	Ser	Asp	Val	Thr	Thr	Ser	Pro	Thr	Pro	Gly	Gly	Ala	2435	2440	2445

-128-

Gly Gly Gly Gln Arg Gly Pro Gly Thr His Met Ser Glu Pro Pro His
 2450 2455 2460

Asn Asn Met Gln Val Tyr Ala
 2465 2470

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2556 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Pro Pro Leu Leu Ala Pro Leu Leu Cys Leu Ala Leu Leu Pro Ala
 1 5 10 15
 Leu Ala Ala Arg Gly Pro Arg Cys Ser Gln Pro Gly Glu Thr Cys Leu
 20 25 30
 Asn Gly Gly Lys Cys Glu Ala Ala Asn Gly Thr Glu Ala Cys Val Cys
 35 40 45
 Gly Gly Ala Phe Val Gly Pro Arg Cys Gln Asp Pro Asn Pro Cys Leu
 50 55 60
 Ser Thr Pro Cys Lys Asn Ala Gly Thr Cys His Val Val Asp Arg Arg
 65 70 75 80
 Gly Val Ala Asp Tyr Ala Cys Ser Cys Ala Leu Gly Phe Ser Gly Pro
 85 90 95
 Leu Cys Leu Thr Pro Leu Asp Asn Ala Cys Leu Thr Asn Pro Cys Arg
 100 105 110
 Asn Gly Gly Thr Cys Asp Leu Leu Thr Leu Thr Glu Tyr Lys Cys Arg
 115 120 125
 Cys Pro Pro Gly Trp Ser Gly Lys Ser Cys Gln Gln Ala Asp Pro Cys
 130 135 140
 Ala Ser Asn Pro Cys Ala Asn Gly Gly Gln Cys Leu Pro Phe Glu Ala
 145 150 155 160
 Ser Tyr Ile Cys His Cys Pro Pro Ser Phe His Gly Pro Thr Cys Arg
 165 170 175
 Gln Asp Val Asn Glu Cys Gly Gln Lys Pro Arg Leu Cys Arg His Gly
 180 185 190
 Gly Thr Cys His Asn Glu Val Gly Ser Tyr Arg Cys Val Cys Arg Ala
 195 200 205
 Thr His Thr Gly Pro Asn Cys Glu Arg Pro Tyr Val Pro Cys Ser Pro
 210 215 220
 Ser Pro Cys Gln Asn Gly Gly Thr Cys Arg Pro Thr Gly Asp Val Thr
 225 230 235 240
 His Glu Cys Ala Cys Leu Pro Gly Phe Thr Gly Gln Asn Cys Glu Glu
 245 250 255

-129-

Asn Ile Asp Asp Cys Pro Gly Asn Asn Cys Lys Asn Gly Gly Ala Cys
 260 265 270
 Val Asp Gly Val Asn Thr Tyr Asn Cys Pro Cys Pro Pro Glu Trp Thr
 275 280 285
 Gly Gln Tyr Cys Thr Glu Asp Val Asp Glu Cys Gln Leu Met Pro Asn
 290 295 300
 Ala Cys Gln Asn Gly Gly Thr Cys His Asn Thr His Gly Gly Tyr Asn
 305 310 315 320
 Cys Val Cys Val Asn Gly Trp Thr Gly Glu Asp Cys Ser Glu Asn Ile
 325 330 335
 Asp Asp Cys Ala Ser Ala Ala Cys Phe His Gly Ala Thr Cys His Asp
 340 345 350
 Arg Val Ala Ser Phe Tyr Cys Glu Cys Pro His Gly Arg Thr Gly Leu
 355 360 365
 Leu Cys His Leu Asn Asp Ala Cys Ile Ser Asn Pro Cys Asn Glu Gly
 370 375 380
 Ser Asn Cys Asp Thr Asn Pro Val Asn Gly Lys Ala Ile Cys Thr Cys
 385 390 395 400
 Pro Ser Gly Tyr Thr Gly Pro Ala Cys Ser Gln Asp Val Asp Glu Cys
 405 410 415
 Ser Leu Gly Ala Asn Pro Cys Glu His Ala Gly Lys Cys Ile Asn Thr
 420 425 430
 Leu Gly Ser Phe Glu Cys Gln Cys Leu Gln Gly Tyr Thr Gly Pro Arg
 435 440 445
 Cys Glu Ile Asp Val Asn Glu Cys Val Ser Asn Pro Cys Gln Asn Asp
 450 455 460
 Ala Thr Cys Leu Asp Gln Ile Gly Glu Phe Gln Cys Met Cys Met Pro
 465 470 475 480
 Gly Tyr Glu Gly Val His Cys Glu Val Asn Thr Asp Glu Cys Ala Ser
 485 490 495
 Ser Pro Cys Leu His Asn Gly Arg Cys Leu Asp Lys Ile Asn Glu Phe
 500 505 510
 Gln Cys Glu Cys Pro Thr Gly Phe Thr Gly His Leu Cys Gln Tyr Asp
 515 520 525
 Val Asp Glu Cys Ala Ser Thr Pro Cys Lys Asn Gly Ala Lys Cys Leu
 530 535 540
 Asp Gly Pro Asn Thr Tyr Thr Cys Val Cys Thr Glu Gly Tyr Thr Gly
 545 550 555 560
 Thr His Cys Glu Val Asp Ile Asp Glu Cys Asp Pro Asp Pro Cys His
 565 570 575
 Tyr Gly Ser Cys Lys Asp Gly Val Ala Thr Phe Thr Cys Leu Cys Arg
 580 585 590
 Pro Gly Tyr Thr Gly His His Cys Glu Thr Asn Ile Asn Glu Cys Ser
 595 600 605
 Ser Gln Pro Cys Arg Leu Arg Gly Thr Cys Gln Asp Pro Asp Asn Ala

-130-

610	615	620
Tyr Leu Cys Phe Cys Leu Lys Gly Thr Thr Gly Pro Asn Cys Glu Ile 625 630 635 640		
Asn Leu Asp Asp Cys Ala Ser Ser Pro Cys Asp Ser Gly Thr Cys Leu 645 650 655		
Asp Lys Ile Asp Gly Tyr Glu Cys Ala Cys Glu Pro Gly Tyr Thr Gly 660 665 670		
Ser Met Cys Asn Ser Asn Ile Asp Glu Cys Ala Gly Asn Pro Cys His 675 680 685		
Asn Gly Gly Thr Cys Glu Asp Gly Ile Asn Gly Phe Thr Cys Arg Cys 690 695 700		
Pro Glu Gly Tyr His Asp Pro Thr Cys Leu Ser Glu Val Asn Glu Cys 705 710 715 720		
Asn Ser Asn Pro Cys Val His Gly Ala Cys Arg Asp Ser Leu Asn Gly 725 730 735		
Tyr Lys Cys Asp Cys Asp Pro Gly Trp Ser Gly Thr Asn Cys Asp Ile 740 745 750		
Asn Asn Asn Glu Cys Glu Ser Asn Pro Cys Val Asn Gly Gly Thr Cys 755 760 765		
Lys Asp Met Thr Ser Gly Ile Val Cys Thr Cys Arg Glu Gly Phe Ser 770 775 780		
Gly Pro Asn Cys Gln Thr Asn Ile Asn Glu Cys Ala Ser Asn Pro Cys 785 790 795 800		
Leu Asn Lys Gly Thr Cys Ile Asp Asp Val Ala Gly Tyr Lys Cys Asn 805 810 815		
Cys Leu Leu Pro Tyr Thr Gly Ala Thr Cys Glu Val Val Leu Ala Pro 820 825 830		
Cys Ala Pro Ser Pro Cys Arg Asn Gly Gly Glu Cys Arg Gln Ser Glu 835 840 845		
Asp Tyr Glu Ser Phe Ser Cys Val Cys Pro Thr Ala Gly Ala Lys Gly 850 855 860		
Gln Thr Cys Glu Val Asp Ile Asn Glu Cys Val Leu Ser Pro Cys Arg 865 870 875 880		
His Gly Ala Ser Cys Gln Asn Thr His Gly Gly Tyr Arg Cys His Cys 885 890 895		
Gln Ala Gly Tyr Ser Gly Arg Asn Cys Glu Thr Asp Ile Asp Asp Cys 900 905 910		
Arg Pro Asn Pro Cys His Asn Gly Gly Ser Cys Thr Asp Gly Ile Asn 915 920 925		
Thr Ala Phe Cys Asp Cys Leu Pro Gly Phe Arg Gly Thr Phe Cys Glu 930 935 940		
Glu Asp Ile Asn Glu Cys Ala Ser Asp Pro Cys Arg Asn Gly Ala Asn 945 950 955 960		
Cys Thr Asp Cys Val Asp Ser Tyr Thr Cys Thr Cys Pro Ala Gly Phe 965 970 975		

-131-

Ser Gly Ile His Cys Glu Asn Asn Thr Pro Asp Cys Thr Glu Ser Ser
 980 985 990
 Cys Phe Asn Gly Gly Thr Cys Val Asp Gly Ile Asn Ser Phe Thr Cys
 995 1000 1005
 Leu Cys Pro Pro Gly Phe Thr Gly Ser Tyr Cys Gln His Val Val Asn
 1010 1015 1020
 Glu Cys Asp Ser Arg Pro Cys Leu Leu Gly Gly Thr Cys Gln Asp Gly
 1025 1030 1035 1040
 Arg Gly Leu His Arg Cys Thr Cys Pro Gln Gly Tyr Thr Gly Pro Asn
 1045 1050 1055
 Cys Gln Asn Leu Val His Trp Cys Asp Ser Ser Pro Cys Lys Asn Gly
 1060 1065 1070
 Gly Lys Cys Trp Gln Thr His Thr Gln Tyr Arg Cys Glu Cys Pro Ser
 1075 1080 1085
 Gly Trp Thr Gly Leu Tyr Cys Asp Val Pro Ser Val Ser Cys Glu Val
 1090 1095 1100
 Ala Ala Gln Arg Gln Gly Val Asp Val Ala Arg Leu Cys Gln His Gly
 1105 1110 1115 1120
 Gly Leu Cys Val Asp Ala Gly Asn Thr His His Cys Arg Cys Gln Ala
 1125 1130 1135
 Gly Tyr Thr Gly Ser Tyr Cys Glu Asp Leu Val Asp Glu Cys Ser Pro
 1140 1145 1150
 Ser Pro Cys Gln Asn Gly Ala Thr Cys Thr Asp Tyr Leu Gly Gly Tyr
 1155 1160 1165
 Ser Cys Lys Cys Val Ala Gly Tyr His Gly Val Asn Cys Ser Glu Glu
 1170 1175 1180
 Ile Asp Glu Cys Leu Ser His Pro Cys Gln Asn Gly Gly Thr Cys Leu
 1185 1190 1195 1200
 Asp Leu Pro Asn Thr Tyr Lys Cys Ser Cys Pro Arg Gly Thr Gln Gly
 1205 1210 1215
 Val His Cys Glu Ile Asn Val Asp Asp Cys Asn Pro Pro Val Asp Pro
 1220 1225 1230
 Val Ser Arg Ser Pro Lys Cys Phe Asn Asn Gly Thr Cys Val Asp Gln
 1235 1240 1245
 Val Gly Gly Tyr Ser Cys Thr Cys Pro Pro Gly Phe Val Gly Glu Arg
 1250 1255 1260
 Cys Glu Gly Asp Val Asn Glu Cys Leu Ser Asn Pro Cys Asp Ala Arg
 1265 1270 1275 1280
 Gly Thr Gln Asn Cys Val Gln Arg Val Asn Asp Phe His Cys Glu Cys
 1285 1290 1295
 Arg Ala Gly His Thr Gly Arg Arg Cys Glu Ser Val Ile Asn Gly Cys
 1300 1305 1310
 Lys Gly Lys Pro Cys Lys Asn Gly Gly Thr Cys Ala Val Ala Ser Asn
 1315 1320 1325
 Thr Ala Arg Gly Phe Ile Cys Lys Cys Pro Ala Gly Phe Glu Gly Ala

-132-

1330	1335	1340
Thr Cys Glu Asn Asp 1345	Ala Arg Thr Cys Gly 1350	Ser Leu Arg Cys Leu Asn 1355 1360
Gly Gly Thr Cys Ile 1365	Ser Gly Pro Arg 1370	Ser Pro Thr Cys Leu Cys Leu 1375
Gly Pro Phe Thr Gly 1380	Pro Glu Cys Gln Phe 1385	Pro Ala Ser Ser Pro Cys 1390
Leu Gly Gly Asn Pro Cys 1395	Tyr Asn Gln Gly Thr 1400	Cys Glu Pro Thr Ser 1405
Glu Ser Pro Phe Tyr 1410	Arg Cys Leu Cys Pro 1415	Ala Lys Phe Asn Gly Leu 1420
Leu Cys His Ile Leu 1425	Asp Tyr Ser Phe Gly 1430	Gly Gly Ala Gly Arg Asp 1435 1440
Ile Pro Pro Pro Leu 1445	Ile Glu Glu Ala Cys 1450	Glu Leu Pro Glu Cys Gln 1455
Glu Asp Ala Gly Asn 1460	Lys Val Cys Ser Leu 1465	Gln Cys Asn Asn His Ala 1470
Cys Gly Trp Asp Gly 1475	Gly Gly Asp Cys Ser 1480	Leu Asn Phe Asn Asp Pro Trp 1485
Lys Asn Cys Thr Gln 1490	Ser Leu Gln Cys Trp 1495	Lys Tyr Phe Ser Asp Gly 1500
His Cys Asp Ser Gln 1505	Cys Asn Ser Ala Gly 1510	Cys Leu Phe Asp Gly Phe 1515 1520
Asp Cys Gln Arg Ala 1525	Glu Gly Gln Cys Asn 1530	Pro Leu Tyr Asp Gln Tyr 1535
Cys Lys Asp His Phe 1540	Ser Asp Gly His Cys 1545	Asp Gln Gly Cys Asn Ser 1550
Ala Glu Cys Glu Trp 1555	Asp Gly Leu Asp Cys 1560	Ala Glu His Val Pro Glu 1565
Arg Leu Ala Ala Gly 1570	Thr Leu Val Val Val 1575	Val Val Leu Met Pro Pro Glu 1580
Gln Leu Arg Asn Ser 1585	Ser Phe His Phe Leu 1590	Arg Glu Leu Ser Arg Val 1595 1600
Leu His Thr Asn Val 1605	Val Phe Lys Arg Asp 1610	Ala His Gly Gln Gln Met 1615
Ile Phe Pro Tyr Tyr 1620	Gly Arg Glu Glu Glu 1625	Leu Arg Lys His Pro Ile 1630
Lys Arg Ala Ala Glu 1635	Gly Trp Ala Ala Pro 1640	Asp Ala Leu Leu Gly Gln 1645
Val Lys Ala Ser Leu 1650	Leu Pro Gly Gly Ser 1655	Glu Gly Gly Arg Arg Arg 1660
Arg Glu Leu Asp Pro 1665	Met Asp Val Arg Gly 1670	Ser Ile Val Tyr Leu Glu 1675 1680
Ile Asp Asn Arg Gln 1685	Cys Val Gln Ala Ser 1690	Ser Ser Gln Cys Phe Gln Ser 1695

-133-

Ala Thr Asp Val Ala Ala Phe Leu Gly Ala Leu Ala Ser Leu Gly Ser
 1700 1705 1710
 Leu Asn Ile Pro Tyr Lys Ile Glu Ala Val Gln Ser Glu Thr Val Glu
 1715 1720 1725
 Pro Pro Pro Pro Ala Gln Leu His Phe Met Tyr Val Ala Ala Ala Ala
 1730 1735 1740
 Phe Val Leu Leu Phe Phe Val Gly Cys Gly Val Leu Leu Ser Arg Lys
 1745 1750 1755 1760
 Arg Arg Arg Gln His Gly Gln Leu Trp Phe Pro Glu Gly Phe Lys Val
 1765 1770 1775
 Ser Glu Ala Ser Lys Lys Lys Arg Arg Glu Glu Leu Gly Glu Asp Ser
 1780 1785 1790
 Val Gly Leu Lys Pro Leu Lys Asn Ala Ser Asp Gly Ala Leu Met Asp
 1795 1800 1805
 Asp Asn Gln Asn Glu Trp Gly Asp Glu Asp Leu Glu Thr Lys Lys Phe
 1810 1815 1820
 Arg Phe Glu Glu Pro Val Val Leu Pro Asp Leu Asp Asp Gln Thr Asp
 1825 1830 1835 1840
 His Arg Gln Trp Thr Gln Gln His Leu Asp Ala Ala Asp Leu Arg Met
 1845 1850 1855
 Ser Ala Met Ala Pro Thr Pro Pro Gln Gly Glu Val Asp Ala Asp Cys
 1860 1865 1870
 Met Asp Val Asn Val Arg Gly Pro Asp Gly Phe Thr Pro Leu Met Ile
 1875 1880 1885
 Ala Ser Cys Ser Gly Gly Gly Leu Glu Thr Gly Asn Ser Glu Glu Glu
 1890 1895 1900
 Glu Asp Ala Pro Ala Val Ile Ser Asp Phe Ile Tyr Gln Gly Ala Ser
 1905 1910 1915 1920
 Leu His Asn Gln Thr Asp Arg Thr Gly Glu Thr Ala Leu His Leu Ala
 1925 1930 1935
 Ala Arg Tyr Ser Arg Ser Asp Ala Ala Lys Arg Leu Leu Glu Ala Ser
 1940 1945 1950
 Ala Asp Ala Asn Ile Gln Asp Asn Met Gly Arg Thr Pro Leu His Ala
 1955 1960 1965
 Ala Val Ser Ala Asp Ala Gln Gly Val Phe Gln Ile Leu Ile Arg Asn
 1970 1975 1980
 Arg Ala Thr Asp Leu Asp Ala Arg Met His Asp Gly Thr Thr Pro Leu
 1985 1990 1995 2000
 Ile Leu Ala Ala Arg Leu Ala Val Glu Gly Met Leu Glu Asp Leu Ile
 2005 2010 2015
 Asn Ser His Ala Asp Val Asn Ala Val Asp Asp Leu Gly Lys Ser Ala
 2020 2025 2030
 Leu His Trp Ala Ala Ala Val Asn Asn Val Asp Ala Ala Val Val Leu
 2035 2040 2045
 Leu Lys Asn Gly Ala Asn Lys Asp Met Gln Asn Asn Arg Glu Glu Thr

-134-

2050	2055	2060
Pro Leu Phe Leu Ala Ala Arg Glu Gly Ser Tyr Glu Thr Ala Lys Val 2065	2070	2075 2080
Leu Leu Asp His Phe Ala Asn Arg Asp Ile Thr Asp His Met Asp Arg 2085	2090	2095
Leu Pro Arg Asp Ile Ala Gln Glu Arg Met His His Asp Ile Val Arg 2100	2105	2110
Leu Leu Asp Glu Tyr Asn Leu Val Arg Ser Pro Gln Leu His Gly Ala 2115	2120	2125
Pro Leu Gly Gly Thr Pro Thr Leu Ser Pro Pro Leu Cys Ser Pro Asn 2130	2135	2140
Gly Tyr Leu Gly Ser Leu Lys Pro Gly Val Gln Gly Lys Lys Val Arg 2145	2150	2155 2160
Lys Pro Ser Ser Lys Gly Leu Ala Cys Gly Ser Lys Glu Ala Lys Asp 2165	2170	2175
Leu Lys Ala Arg Arg Lys Lys Ser Gln Asp Gly Lys Gly Cys Leu Leu 2180	2185	2190
Asp Ser Ser Gly Met Leu Ser Pro Val Asp Ser Leu Glu Ser Pro His 2195	2200	2205
Gly Tyr Leu Ser Asp Val Ala Ser Pro Pro Leu Leu Pro Ser Pro Phe 2210	2215	2220
Gln Gln Ser Pro Ser Val Pro Leu Asn His Leu Pro Gly Met Pro Asp 2225	2230	2235 2240
Thr His Leu Gly Ile Gly His Leu Asn Val Ala Ala Lys Pro Glu Met 2245	2250	2255
Ala Ala Leu Gly Gly Gly Gly Arg Leu Ala Phe Glu Thr Gly Pro Pro 2260	2265	2270
Arg Leu Ser His Leu Pro Val Ala Ser Gly Thr Ser Thr Val Leu Gly 2275	2280	2285
Ser Ser Ser Gly Gly Ala Leu Asn Phe Thr Val Gly Gly Ser Thr Ser 2290	2295	2300
Leu Asn Gly Gln Cys Glu Trp Leu Ser Arg Leu Gln Ser Gly Met Val 2305	2310	2315 2320
Pro Asn Gln Tyr Asn Pro Leu Arg Gly Ser Val Ala Pro Gly Pro Leu 2325	2330	2335
Ser Thr Gln Ala Pro Ser Leu Gln His Gly Met Val Gly Pro Leu His 2340	2345	2350
Ser Ser Leu Ala Ala Ser Ala Leu Ser Gln Met Met Ser Tyr Gln Gly 2355	2360	2365
Leu Pro Ser Thr Arg Leu Ala Thr Gln Pro His Leu Val Gln Thr Gln 2370	2375	2380
Gln Val Gln Pro Gln Asn Leu Gln Met Gln Gln Gln Asn Leu Gln Pro 2385	2390	2395 2400
Ala Asn Ile Gln Gln Gln Gln Ser Leu Gln Pro Pro Pro Pro Pro Pro 2405	2410	2415

-135-

Gln Pro His Leu Gly Val Ser Ser Ala Ala Ser Gly His Leu Gly Arg
 2420 2425 2430

Ser Phe Leu Ser Gly Glu Pro Ser Gln Ala Asp Val Gln Pro Leu Gly
 2435 2440 2445

Pro Ser Ser Leu Ala Val His Thr Ile Leu Pro Gln Glu Ser Pro Ala
 2450 2455 2460

Leu Pro Thr Ser Leu Pro Ser Ser Leu Val Pro Pro Val Thr Ala Ala
 2465 2470 2475 2480

Gln Phe Leu Thr Pro Pro Ser Gln His Ser Tyr Ser Ser Pro Val Glu
 2485 2490 2495

Asn Thr Pro Ser His Gln Leu Gln Val Pro Glu His Pro Phe Leu Thr
 2500 2505 2510

Pro Ser Pro Glu Ser Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser
 2515 2520 2525

Asn Val Ser Asp Trp Ser Glu Gly Val Ser Ser Pro Pro Thr Ser Met
 2530 2535 2540

Gln Ser Gln Ile Ala Arg Ile Pro Glu Ala Phe Lys
 2545 2550 2555

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9723 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 10..7419

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGAATTCCG CCC GCC CTG CGC CCC GCT CTG CTG TGG GCG CTG CTG GCG	48
Pro Ala Leu Arg Pro Ala Leu Leu Trp Ala Leu Leu Ala	
1 5 10	
CTC TGG CTG TGC TGC GCG GCC CCC GCG CAT GCA TTG CAG TGT CGA GAT	96
Leu Trp Leu Cys Cys Ala Ala Pro Ala His Ala Leu Gln Cys Arg Asp	
15 20 25	
GGC TAT GAA CCC TGT GTA AAT GAA GGA ATG TGT GTT ACC TAC CAC AAT	144
Gly Tyr Glu Pro Cys Val Asn Glu Gly Met Cys Val Thr Tyr His Asn	
30 35 40 45	
GGC ACA GGA TAC TGC AAA TGT CCA GAA GGC TTC TTG GGG GAA TAT TGT	192
Gly Thr Gly Tyr Cys Lys Cys Pro Glu Gly Phe Leu Gly Glu Tyr Cys	
50 55 60	
CAA CAT CGA GAC CCC TGT GAG AAG AAC CGC TGC CAG AAT GGT GGG ACT	240
Gln His Arg Asp Pro Cys Glu Lys Asn Arg Cys Gln Asn Gly Gly Thr	
65 70 75	
TGT GTG GCC CAG GCC ATG CTG GGG AAA GCC ACG TGC CGA TGT GCC TCA	288
Cys Val Ala Gln Ala Met Leu Gly Lys Ala Thr Cys Arg Cys Ala Ser	

-136-

80	85	90	
GGG TTT ACA GGA GAG GAC TGC CAG TAC TCA ACA TCT CAT CCA TGC TTT Gly Phe Thr Gly Glu Asp Cys Gln Tyr Ser Thr Ser His Pro Cys Phe 95 100 105			336
GTG TCT CGA CCC TGC CTG AAT GGC GGC ACA TGC CAT ATG CTC AGC CGG Val Ser Arg Pro Cys Leu Asn Gly Gly Thr Cys His Met Leu Ser Arg 110 115 120 125			384
GAT ACC TAT GAG TGC ACC TGT CAA GTC GGG TTT ACA GGT AAG GAG TGC Asp Thr Tyr Glu Cys Thr Cys Gln Val Gly Phe Thr Gly Lys Glu Cys 130 135 140			432
CAA TGG ACG GAT GCC TGC CTG TCT CAT CCC TGT GCA AAT GGA AGT ACC Gln Trp Thr Asp Ala Cys Leu Ser His Pro Cys Ala Asn Gly Ser Thr 145 150 155			480
TGT ACC ACT GTG GCC AAC CAG TTC TCC TGC AAA TGC CTC ACA GGC TTC Cys Thr Thr Val Ala Asn Gln Phe Ser Cys Lys Cys Leu Thr Gly Phe 160 165 170			528
ACA GGG CAG AAA TGT GAG ACT GAT GTC AAT GAG TGT GAC ATT CCA GGA Thr Gly Gln Lys Cys Glu Thr Asp Val Asn Glu Cys Asp Ile Pro Gly 175 180 185			576
CAC TGC CAG CAT GGT GGC ACC TGC CTC AAC CTG CCT GGT TCC TAC CAG His Cys Gln His Gly Gly Thr Cys Leu Asn Leu Pro Gly Ser Tyr Gln 190 195 200 205			624
TGC CAG TGC CCT CAG GGC TTC ACA GGC CAG TAC TGT GAC AGC CTG TAT Cys Gln Cys Pro Gln Gly Phe Thr Gly Gln Tyr Cys Asp Ser Leu Tyr 210 215 220			672
GTG CCC TGT GCA CCC TCA CCT TGT GTC AAT GGA GGC ACC TGT CGG CAG Val Pro Cys Ala Pro Ser Pro Cys Val Asn Gly Gly Thr Cys Arg Gln 225 230 235			720
ACT GGT GAC TTC ACT TTT GAG TGC AAC TGC CTT CCA GGT TTT GAA GGG Thr Gly Asp Phe Thr Phe Glu Cys Asn Cys Leu Pro Gly Phe Glu Gly 240 245 250			768
AGC ACC TGT GAG AGG AAT ATT GAT GAC TGC CCT AAC CAC AGG TGT CAG Ser Thr Cys Glu Arg Asn Ile Asp Asp Cys Pro Asn His Arg Cys Gln 255 260 265			816
AAT GGA GGG GTT TGT GTG GAT GGG GTC AAC ACT TAC AAC TGC CGC TGT Asn Gly Gly Val Cys Val Asp Gly Val Asn Thr Tyr Asn Cys Arg Cys 270 275 280 285			864
CCC CCA CAA TGG ACA GGA CAG TTC TGC ACA GAG GAT GTG GAT GAA TGC Pro Pro Gln Trp Thr Gly Gln Phe Cys Thr Glu Asp Val Asp Glu Cys 290 295 300			912
CTG CTG CAG CCC AAT GCC TGT CAA AAT GGG GGC ACC TGT GCC AAC CGC Leu Leu Gln Pro Asn Ala Cys Gln Asn Gly Gly Thr Cys Ala Asn Arg 305 310 315			960
AAT GGA GGC TAT GGC TGT GTA TGT GTC AAC GGC TGG AGT GGA GAT GAC Asn Gly Gly Tyr Gly Cys Val Cys Val Asn Gly Trp Ser Gly Asp Asp 320 325 330			1008
TGC AGT GAG AAC ATT GAT GAT TGT GCC TTC GCC TCC TGT ACT CCA GGC Cys Ser Glu Asn Ile Asp Asp Cys Ala Phe Ala Ser Cys Thr Pro Gly 335 340 345			1056
TCC ACC TGC ATC GAC CGT GTG GCC TCC TTC TCT TGC ATG TGC CCA GAG			1104

-137-

Ser Thr Cys Ile Asp Arg Val Ala Ser Phe Ser Cys Met Cys Pro Glu	
350 355 360 365	
GGG AAG GCA GGT CTC CTG TGT CAT CTG GAT GAT GCA TGC ATC AGC AAT	1152
Gly Lys Ala Gly Leu Leu Cys His Leu Asp Asp Ala Cys Ile Ser Asn	
370 375 380	
CCT TGC CAC AAG GGG GCA CTG TGT GAC ACC AAC CCC CTA AAT GGG CAA	1200
Pro Cys His Lys Gly Ala Leu Cys Asp Thr Asn Pro Leu Asn Gly Gln	
385 390 395	
TAT ATT TGC ACC TGC CCA CAA GGC TAC AAA GGG GCT GAC TGC ACA GAA	1248
Tyr Ile Cys Thr Cys Pro Gln Gly Tyr Lys Gly Ala Asp Cys Thr Glu	
400 405 410	
GAT GTG GAT GAA TGT GCC ATG GCC AAT AGC AAT CCT TGT GAG CAT GCA	1296
Asp Val Asp Glu Cys Ala Met Ala Asn Ser Asn Pro Cys Glu His Ala	
415 420 425	
GGA AAA TGT GTG AAC ACG GAT GGC GCC TTC CAC TGT GAG TGT CTG AAG	1344
Gly Lys Cys Val Asn Thr Asp Gly Ala Phe His Cys Glu Cys Leu Lys	
430 435 440 445	
GGT TAT GCA GGA CCT CGT TGT GAG ATG GAC ATC AAT GAG TGC CAT TCA	1392
Gly Tyr Ala Gly Pro Arg Cys Glu Met Asp Ile Asn Glu Cys His Ser	
450 455 460	
GAC CCC TGC CAG AAT GAT GCT ACC TGT CTG GAT AAG ATT GGA GGC TTC	1440
Asp Pro Cys Gln Asn Asp Ala Thr Cys Leu Asp Lys Ile Gly Gly Phe	
465 470 475	
ACA TGT CTG TGC ATG CCA GGT TTC AAA GGT GTG CAT TGT GAA TTA GAA	1488
Thr Cys Leu Cys Met Pro Gly Phe Lys Gly Val His Cys Glu Leu Glu	
480 485 490	
ATA AAT GAA TGT CAG AGC AAC CCT TGT GTG AAC AAT GGG CAG TGT GTG	1536
Ile Asn Glu Cys Gln Ser Asn Pro Cys Val Asn Gly Gln Cys Val	
495 500 505	
GAT AAA GTC AAT CGT TTC CAG TGC CTG TGT CCT CCT GGT TTC ACT GGG	1584
Asp Lys Val Asn Arg Phe Gln Cys Leu Cys Pro Pro Gly Phe Thr Gly	
510 515 520 525	
CCA GTT TGC CAG ATT GAT ATT GAT GAC TGT TCC AGT ACT CCG TGT CTG	1632
Pro Val Cys Gln Ile Asp Ile Asp Asp Cys Ser Ser Thr Pro Cys Leu	
530 535 540	
AAT GGG GCA AAG TGT ATC GAT CAC CCG AAT GGC TAT GAA TGC CAG TGT	1680
Asn Gly Ala Lys Cys Ile Asp His Pro Asn Gly Tyr Glu Cys Gln Cys	
545 550 555	
GCC ACA GGT TTC ACT GGT GTG TTG TGT GAG GAG AAC ATT GAC AAC TGT	1728
Ala Thr Gly Phe Thr Gly Val Leu Cys Glu Glu Asn Ile Asp Asn Cys	
560 565 570	
GAC CCC GAT CCT TGC CAC CAT GGT CAG TGT CAG GAT GGT ATT GAT TCC	1776
Asp Pro Asp Pro Cys His His Gly Gln Cys Gln Asp Gly Ile Asp Ser	
575 580 585	
TAC ACC TGC ATC TGC AAT CCC GGG TAC ATG GGC GCC ATC TGC AGT GAC	1824
Tyr Thr Cys Ile Cys Asn Pro Gly Tyr Met Gly Ala Ile Cys Ser Asp	
590 595 600 605	
CAG ATT GAT GAA TGT TAC AGC AGC CCT TGC CTG AAC GAT GGT CGC TGC	1872
Gln Ile Asp Glu Cys Tyr Ser Ser Pro Cys Leu Asn Asp Gly Arg Cys	
610 615 620	

-138-

ATT GAC CTG GTC AAT GGC TAC CAG TGC AAC TGC CAG CCA GGC ACG TCA Ile Asp Leu Val Asn Gly Tyr Gln Cys Asn Cys Gln Pro Gly Thr Ser 625 630 635	1920
GGG GTT AAT TGT GAA ATT AAT TTT GAT GAC TGT GCA AGT AAC CCT TGT Gly Val Asn Cys Glu Ile Asn Phe Asp Asp Cys Ala Ser Asn Pro Cys 640 645 650	1968
ATC CAT GGA ATC TGT ATG GAT GGC ATT AAT CGC TAC AGT TGT GTC TGC Ile His Gly Ile Cys Met Asp Gly Ile Asn Arg Tyr Ser Cys Val Cys 655 660 665	2016
TCA CCA GGA TTC ACA GGG CAG AGA TGT AAC ATT GAC ATT GAT GAG TGT Ser Pro Gly Phe Thr Gly Gln Arg Cys Asn Ile Asp Ile Asp Glu Cys 670 675 680 685	2064
GCC TCC AAT CCC TGT CGC AAG GGT GCA ACA TGT ATC AAC GGT GTG AAT Ala Ser Asn Pro Cys Arg Lys Gly Ala Thr Cys Ile Asn Gly Val Asn 690 695 700	2112
GGT TTC CGC TGT ATA TGC CCC GAG GGA CCC CAT CAC CCC AGC TGC TAC Gly Phe Arg Cys Ile Cys Pro Glu Gly Pro His His Pro Ser Cys Tyr 705 710 715	2160
TCA CAG GTG AAC GAA TGC CTG AGC AAT CCC TGC ATC CAT GGA AAC TGT Ser Gln Val Asn Glu Cys Leu Ser Asn Pro Cys Ile His Gly Asn Cys 720 725 730	2208
ACT GGA GGT CTC AGT GGA TAT AAG TGT CTC TGT GAT GCA GGC TGG GTT Thr Gly Gly Leu Ser Gly Tyr Lys Cys Leu Cys Asp Ala Gly Trp Val 735 740 745	2256
GGC ATC AAC TGT GAA GTG GAC AAA AAT GAA TGC CTT TCG AAT CCA TGC Gly Ile Asn Cys Glu Val Asp Lys Asn Glu Cys Leu Ser Asn Pro Cys 750 755 760 765	2304
CAG AAT GGA GGA ACT TGT GAC AAT CTG GTG AAT GGA TAC AGG TGT ACT Gln Asn Gly Gly Thr Cys Asp Asn Leu Val Asn Gly Tyr Arg Cys Thr 770 775 780	2352
TGC AAG AAG GGC TTT AAA GGC TAT AAC TGC CAG GTG AAT ATT GAT GAA Cys Lys Lys Gly Phe Lys Gly Tyr Asn Cys Gln Val Asn Ile Asp Glu 785 790 795	2400
TGT GCC TCA AAT CCA TGC CTG AAC CAA GGA ACC TGC TTT GAT GAC ATA Cys Ala Ser Asn Pro Cys Leu Asn Gln Gly Thr Cys Phe Asp Asp Ile 800 805 810	2448
AGT GGC TAC ACT TGC CAC TGT GTG CTG CCA TAC ACA GGC AAG AAT TGT Ser Gly Tyr Thr Cys His Cys Val Leu Pro Tyr Thr Gly Lys Asn Cys 815 820 825	2496
CAG ACA GTA TTG GCT CCC TGT TCC CCA AAC CCT TGT GAG AAT GCT GCT Gln Thr Val Leu Ala Pro Cys Ser Pro Asn Pro Cys Glu Asn Ala Ala 830 835 840 845	2544
GTT TGC AAA GAG TCA CCA AAT TTT GAG AGT TAT ACT TGC TTG TGT GCT Val Cys Lys Glu Ser Pro Asn Phe Glu Ser Tyr Thr Cys Leu Cys Ala 850 855 860	2592
CCT GGC TGG CAA GGT CAG CGG TGT ACC ATT GAC ATT GAC GAG TGT ATC Pro Gly Trp Gln Gly Gln Arg Cys Thr Ile Asp Ile Asp Glu Cys Ile 865 870 875	2640
TCC AAG CCC TGC ATG AAC CAT GGT CTC TGC CAT AAC ACC CAG GGC AGC Ser Lys Pro Cys Met Asn His Gly Leu Cys His Asn Thr Gln Gly Ser 880 885 890	2688

-139-

TAC ATG TGT GAA TGT CCA CCA GGC TTC AGT GGT ATG GAC TGT GAG GAG Tyr Met Cys Glu Cys Pro Pro Gly Phe Ser Gly Met Asp Cys Glu Glu 895 900 905	2736
GAC ATT GAT GAC TGC CTT GCC AAT CCT TGC CAG AAT GGA GGT TCC TGT Asp Ile Asp Asp Cys Leu Ala Asn Pro Cys Gln Asn Gly Gly Ser Cys 910 915 920 925	2784
ATG GAT GGA GTG AAT ACT TTC TCC TGC CTC TGC CTT CCG GGT TTC ACT Met Asp Gly Val Asn Thr Phe Ser Cys Leu Cys Leu Pro Gly Phe Thr 930 935 940	2832
GGG GAT AAG TGC CAG ACA GAC ATG AAT GAG TGT CTG AGT GAA CCC TGT Gly Asp Lys Cys Gln Thr Asp Met Asn Glu Cys Leu Ser Glu Pro Cys 945 950 955	2880
AAG AAT GGA GGG ACC TGC TCT GAC TAC GTC AAC AGT TAC ACT TGC AAG Lys Asn Gly Gly Thr Cys Ser Asp Tyr Val Asn Ser Tyr Thr Cys Lys 960 965 970	2928
TGC CAG GCA GGA TTT GAT GGA GTC CAT TGT GAG AAC AAC ATC AAT GAG Cys Gln Ala Gly Phe Asp Gly Val His Cys Glu Asn Asn Ile Asn Glu 975 980 985	2976
TGC ACT GAG AGC TCC TGT TTC AAT GGT GGC ACA TGT GTT GAT GGG ATT Cys Thr Glu Ser Ser Cys Phe Asn Gly Gly Thr Cys Val Asp Gly Ile 990 995 1000 1005	3024
AAC TCC TTC TCT TGC TTG TGC CCT GTG GGT TTC ACT GGA TCC TTC TGC Asn Ser Phe Ser Cys Leu Cys Pro Val Gly Phe Thr Gly Ser Phe Cys 1010 1015 1020	3072
CTC CAT GAG ATC AAT GAA TGC AGC TCT CAT CCA TGC CTG AAT GAG GGA Leu His Glu Ile Asn Glu Cys Ser Ser His Pro Cys Leu Asn Glu Gly 1025 1030 1035	3120
ACG TGT GTT GAT GGC CTG GGT ACC TAC CGC TGC AGC TGC CCC CTG GGC Thr Cys Val Asp Gly Leu Gly Thr Tyr Arg Cys Ser Cys Pro Leu Gly 1040 1045 1050	3168
TAC ACT GGG AAA AAC TGT CAG ACC CTG GTG AAT CTC TGC AGT CGG TCT Tyr Thr Gly Lys Asn Cys Gln Thr Leu Val Asn Leu Cys Ser Arg Ser 1055 1060 1065	3216
CCA TGT AAA AAC AAA GGT ACT TGT GTT CAG AAA AAA GCA GAG TCC CAG Pro Cys Lys Asn Lys Gly Thr Cys Val Gln Lys Lys Ala Glu Ser Gln 1070 1075 1080 1085	3264
TGC CTA TGT CCA TCT GGA TGG GCT GGT GCC TAT TGT GAC GTG CCC AAT Cys Leu Cys Pro Ser Gly Trp Ala Gly Ala Tyr Cys Asp Val Pro Asn 1090 1095 1100	3312
GTC TCT TGT GAC ATA GCA GCC TCC AGG AGA GGT GTG CTT GTT GAA CAC Val Ser Cys Asp Ile Ala Ala Ser Arg Arg Gly Val Leu Val Glu His 1105 1110 1115	3360
TTG TGC CAG CAC TCA GGT GTC TGC ATC AAT GCT GGC AAC ACG CAT TAC Leu Cys Gln His Ser Gly Val Cys Ile Asn Ala Gly Asn Thr His Tyr 1120 1125 1130	3408
TGT CAG TGC CCC CTG GGC TAT ACT GGG AGC TAC TGT GAG GAG CAA CTC Cys Gln Cys Pro Leu Gly Tyr Thr Gly Ser Tyr Cys Glu Glu Gln Leu 1135 1140 1145	3456
GAT GAG TGT GCG TCC AAC CCC TGC CAG CAC GGG GCA ACA TGC AGT GAC Asp Glu Cys Ala Ser Asn Pro Cys Gln His Gly Ala Thr Cys Ser Asp 1150 1155 1160 1165	3504

-140-

TTC ATT GGT GGA TAC AGA TGC GAG TGT GTC CCA GGC TAT CAG GGT GTC	3552
Phe Ile Gly Gly Tyr Arg Cys Glu Cys Val Pro Gly Tyr Gln Gly Val	
1170 1175 1180	
AAC TGT GAG TAT GAA GTG GAT GAG TGC CAG AAT CAG CCC TGC CAG AAT	3600
Asn Cys Glu Tyr Glu Val Asp Glu Cys Gln Asn Gln Pro Cys Gln Asn	
1185 1190 1195	
GGA GGC ACC TGT ATT GAC CTT GTG AAC CAT TTC AAG TGC TCT TGC CCA	3648
Gly Gly Thr Cys Ile Asp Leu Val Asn His Phe Lys Cys Ser Cys Pro	
1200 1205 1210	
CCA GGC ACT CGG GGC CTA CTC TGT GAA GAG AAC ATT GAT GAC TGT GCC	3696
Pro Gly Thr Arg Gly Leu Leu Cys Glu Glu Asn Ile Asp Asp Cys Ala	
1215 1220 1225	
CGG GGT CCC CAT TGC CTT AAT GGT GGT CAG TGC ATG GAT AGG ATT GGA	3744
Arg Gly Pro His Cys Leu Asn Gly Gly Gln Cys Met Asp Arg Ile Gly	
1230 1235 1240 1245	
GGC TAC AGT TGT CGC TGC TTG CCT GGC TTT GCT GGG GAG CGT TGT GAG	3792
Gly Tyr Ser Cys Arg Cys Leu Pro Gly Phe Ala Gly Glu Arg Cys Glu	
1250 1255 1260	
GGA GAC ATC AAC GAG TGC CTC TCC AAC CCC TGC AGC TCT GAG GGC AGC	3840
Gly Asp Ile Asn Glu Cys Leu Ser Asn Pro Cys Ser Ser Glu Gly Ser	
1265 1270 1275	
CTG GAC TGT ATA CAG CTC ACC AAT GAC TAC CTG TGT GTT TGC CGT AGT	3888
Leu Asp Cys Ile Gln Leu Thr Asn Asp Tyr Leu Cys Val Cys Arg Ser	
1280 1285 1290	
GCC TTT ACT GGC CGG CAC TGT GAA ACC TTC GTC GAT GTG TGT CCC CAG	3936
Ala Phe Thr Gly Arg His Cys Glu Thr Phe Val Asp Val Cys Pro Gln	
1295 1300 1305	
ATG CCC TGC CTG AAT GGA GGG ACT TGT GCT GTG GCC AGT AAC ATG CCT	3984
Met Pro Cys Leu Asn Gly Gly Thr Cys Ala Val Ala Ser Asn Met Pro	
1310 1315 1320 1325	
GAT GGT TTC ATT TGC CGT TGT CCC CCG GGA TTT TCC GGG GCA AGG TGC	4032
Asp Gly Phe Ile Cys Arg Cys Pro Pro Gly Phe Ser Gly Ala Arg Cys	
1330 1335 1340	
CAG AGC AGC TGT GGA CAA GTG AAA TGT AGG AAG GGG GAG CAG TGT GTG	4080
Gln Ser Ser Cys Gly Gln Val Lys Cys Arg Lys Gly Glu Gln Cys Val	
1345 1350 1355	
CAC ACC GCC TCT GGA CCC CGC TGC TTC TGC CCC AGT CCC CGG GAC TGC	4128
His Thr Ala Ser Gly Pro Arg Cys Phe Cys Pro Ser Pro Arg Asp Cys	
1360 1365 1370	
GAG TCA GGC TGT GCC AGT AGC CCC TGC CAG CAC GGG GGC AGC TGC CAC	4176
Glu Ser Gly Cys Ala Ser Ser Pro Cys Gln His Gly Gly Ser Cys His	
1375 1380 1385	
CCT CAG CGC CAG CCT CCT TAT TAC TCC TGC CAG TGT GCC CCA CCA TTC	4224
Pro Gln Arg Gln Pro Pro Tyr Tyr Ser Cys Gln Cys Ala Pro Pro Phe	
1390 1395 1400 1405	
TCG GGT AGC CGC TGT GAA CTC TAC ACG GCA CCC CCC AGC ACC CCT CCT	4272
Ser Gly Ser Arg Cys Glu Leu Tyr Thr Ala Pro Pro Ser Thr Pro Pro	
1410 1415 1420	
GCC ACC TGT CTG AGC CAG TAT TGT GCC GAC AAA GCT CGG GAT GGC GTC	4320
Ala Thr Cys Leu Ser Gln Tyr Cys Ala Asp Lys Ala Arg Asp Gly Val	
1425 1430 1435	

-141-

TGT GAT GAG GCC TGC AAC AGC CAT GCC TGC CAG TGG GAT GGG GGT GAC Cys Asp Glu Ala Cys Asn Ser His Ala Cys Gln Trp Asp Gly Gly Asp 1440 1445 1450	4368
TGT TCT CTC ACC ATG GAG AAC CCC TGG GCC AAC TGC TCC TCC CCA CTT Cys Ser Leu Thr Met Glu Asn Pro Trp Ala Asn Cys Ser Ser Pro Leu 1455 1460 1465	4416
CCC TGC TGG GAT TAT ATC AAC AAC CAG TGT GAT GAG CTG TGC AAC ACG Pro Cys Trp Asp Tyr Ile Asn Asn Gln Cys Asp Glu Leu Cys Asn Thr 1470 1475 1480 1485	4464
GTC GAG TGC CTG TTT GAC AAC TTT GAA TGC CAG GGG AAC AGC AAG ACA Val Glu Cys Leu Phe Asp Asn Phe Glu Cys Gln Gly Asn Ser Lys Thr 1490 1495 1500	4512
TGC AAG TAT GAC AAA TAC TGT GCA GAC CAC TTC AAA GAC AAC CAC TGT Cys Lys Tyr Asp Lys Tyr Cys Ala Asp His Phe Lys Asp Asn His Cys 1505 1510 1515	4560
AAC CAG GGG TGC AAC AGT GAG GAG TGT GGT TGG GAT GGG CTG GAC TGT Asn Gln Gly Cys Asn Ser Glu Glu Cys Gly Trp Asp Gly Leu Asp Cys 1520 1525 1530	4608
GCT GCT GAC CAA CCT GAG AAC CTG GCA GAA GGT ACC CTG GTT ATT GTG Ala Ala Asp Gln Pro Glu Asn Leu Ala Glu Gly Thr Leu Val Ile Val 1535 1540 1545	4656
GTA TTG ATG CCA CCT GAA CAA CTG CTC CAG GAT GCT CGC AGC TTC TTG Val Leu Met Pro Pro Glu Gln Leu Leu Gln Asp Ala Arg Ser Phe Leu 1550 1555 1560 1565	4704
CGG GCA CTG GGT ACC CTG CTC CAC ACC AAC CTG CGC ATT AAG CGG GAC Arg Ala Leu Gly Thr Leu Leu His Thr Asn Leu Arg Ile Lys Arg Asp 1570 1575 1580	4752
TCC CAG GGG GAA CTC ATG GTG TAC CCC TAT TAT GGT GAG AAG TCA GCT Ser Gln Gly Glu Leu Met Val Tyr Pro Tyr Tyr Gly Glu Lys Ser Ala 1585 1590 1595	4800
GCT ATG AAG AAA CAG AGG ATG ACA CGC AGA TCC CTT CCT GGT GAA CAA Ala Met Lys Lys Gln Arg Met Thr Arg Arg Ser Leu Pro Gly Glu Gln 1600 1605 1610	4848
GAA CAG GAG GTG GCT GGC TCT AAA GTC TTT CTG GAA ATT GAC AAC CGC Glu Gln Glu Val Ala Gly Ser Lys Val Phe Leu Glu Ile Asp Asn Arg 1615 1620 1625	4896
CAG TGT GTT CAA GAC TCA GAC CAC TGC TTC AAG AAC ACG GAT GCA GCA Gln Cys Val Gln Asp Ser Asp His Cys Phe Lys Asn Thr Asp Ala Ala 1630 1635 1640 1645	4944
GCA GCT CTC CTG GCC TCT CAC GCC ATA CAG GGG ACC CTG TCA TAC CCT Ala Ala Leu Leu Ala Ser His Ala Ile Gln Gly Thr Leu Ser Tyr Pro 1650 1655 1660	4992
CTT GTG TCT GTC GTC AGT GAA TCC CTG ACT CCA GAA CGC ACT CAG CTC Leu Val Ser Val Val Ser Glu Ser Leu Thr Pro Glu Arg Thr Gln Leu 1665 1670 1675	5040
CTC TAT CTC CTT GCT GTT GCT GTT GTC ATC ATT CTG TTT ATT ATT CTG Leu Tyr Leu Leu Ala Val Ala Val Val Ile Ile Leu Phe Ile Ile Leu 1680 1685 1690	5088
CTG GGG GTA ATC ATG GCA AAA CGA AAG CGT AAG CAT GGC TCT CTC TGG Leu Gly Val Ile Met Ala Lys Arg Lys Arg Lys His Gly Ser Leu Trp 1695 1700 1705	5136

-142-

CTG CCT GAA GGT TTC ACT CTT CGC CGA GAT GCA AGC AAT CAC AAG CGT Leu Pro Glu Gly Phe Thr Leu Arg Arg Asp Ala Ser Asn His Lys Arg 1710 1715 1720 1725	5184
CGT GAG CCA GTG GGA CAG GAT GCT GTG GGG CTG AAA AAT CTC TCA GTG Arg Glu Pro Val Gly Gln Asp Ala Val Gly Leu Lys Asn Leu Ser Val 1730 1735 1740	5232
CAA GTC TCA GAA GCT AAC CTA ATT GGT ACT GGA ACA AGT GAA CAC TGG Gln Val Ser Glu Ala Asn Leu Ile Gly Thr Gly Thr Ser Glu His Trp 1745 1750 1755	5280
GTC GAT GAT GAA GGG CCC CAG CCA AAG AAA GTA AAG GCT GAA GAT GAG Val Asp Asp Glu Gly Pro Gln Pro Lys Lys Val Lys Ala Glu Asp Glu 1760 1765 1770	5328
GCC TTA CTC TCA GAA GAA GAT GAC CCC ATT GAT CGA CGG CCA TGG ACA Ala Leu Leu Ser Glu Glu Asp Asp Pro Ile Asp Arg Arg Pro Trp Thr 1775 1780 1785	5376
CAG CAG CAC CTT GAA GCT GCA GAC ATC CGT AGG ACA CCA TCG CTG GCT Gln Gln His Leu Glu Ala Ala Asp Ile Arg Arg Thr Pro Ser Leu Ala 1790 1795 1800 1805	5424
CTC ACC CCT CCT CAG GCA GAG CAG GAG GTG GAT GTG TTA GAT GTG AAT Leu Thr Pro Pro Gln Ala Glu Gln Glu Val Asp Val Leu Asp Val Asn 1810 1815 1820	5472
GTC CGT GGC CCA GAT GGC TGC ACC CCA TTG ATG TTG GCT TCT CTC CGA Val Arg Gly Pro Asp Gly Cys Thr Pro Leu Met Leu Ala Ser Leu Arg 1825 1830 1835	5520
GGA GGC AGC TCA GAT TTG AGT GAT GAA GAT GAA GAT GCA GAG GAC TCT Gly Gly Ser Ser Asp Leu Ser Asp Glu Asp Glu Asp Ala Glu Asp Ser 1840 1845 1850	5568
TCT GCT AAC ATC ATC ACA GAC TTG GTC TAC CAG GGT GCC AGC CTC CAG Ser Ala Asn Ile Ile Thr Asp Leu Val Tyr Gln Gly Ala Ser Leu Gln 1855 1860 1865	5616
GCC CAG ACA GAC CGG ACT GGT GAG ATG GCC CTG CAC CTT GCA GCC CGC Ala Gln Thr Asp Arg Thr Gly Glu Met Ala Leu His Leu Ala Ala Arg 1870 1875 1880 1885	5664
TAC TCA CGG GCT GAT GCT GCC AAG CGT CTC CTG GAT GCA GGT GCA GAT Tyr Ser Arg Ala Asp Ala Ala Lys Arg Leu Leu Asp Ala Gly Ala Asp 1890 1895 1900	5712
GCC AAT GCC CAG GAC AAC ATG GGC CGC TGT CCA CTC CAT GCT GCA GTG Ala Asn Ala Gln Asp Asn Met Gly Arg Cys Pro Leu His Ala Ala Val 1905 1910 1915	5760
GCA GCT GAT GCC CAA GGT GTC TTC CAG ATT CTG ATT CGC AAC CGA GTA Ala Ala Asp Ala Gln Gly Val Phe Gln Ile Leu Ile Arg Asn Arg Val 1920 1925 1930	5808
ACT GAT CTA GAT GCC AGG ATG AAT GAT GGT ACT ACA CCC CTG ATC CTG Thr Asp Leu Asp Ala Arg Met Asn Asp Gly Thr Thr Pro Leu Ile Leu 1935 1940 1945	5856
GCT GCC CGC CTG GCT GTG GAG GGA ATG GTG GCA GAA CTG ATC AAC TGC Ala Ala Arg Leu Ala Val Glu Gly Met Val Ala Glu Leu Ile Asn Cys 1950 1955 1960 1965	5904
CAA GCG GAT GTG AAT GCA GTG GAT GAC CAT GGA AAA TCT GCT CTT CAC Gln Ala Asp Val Asn Ala Val Asp Asp His Gly Lys Ser Ala Leu His 1970 1975 1980	5952

-143-

TGG	GCA	GCT	GCT	GTC	AAT	AAT	GTG	GAG	GCA	ACT	CTT	TTG	TTG	TTG	AAA	6000
Trp	Ala	Ala	Ala	Val	Asn	Asn	Val	Glu	Ala	Thr	Leu	Leu	Leu	Leu	Lys	
			1985					1990						1995		
AAT	GGG	GCC	AAC	CGA	GAC	ATG	CAG	GAC	AAC	AAG	GAA	GAG	ACA	CCT	CTG	6048
Asn	Gly	Ala	Asn	Arg	Asp	Met	Gln	Asp	Asn	Lys	Glu	Glu	Thr	Pro	Leu	
		2000					2005					2010				
TTT	CTT	GCT	GCC	CGG	GAG	GGG	AGC	TAT	GAA	GCA	GCC	AAG	ATC	CTG	TTA	6096
Phe	Leu	Ala	Ala	Arg	Glu	Gly	Ser	Tyr	Glu	Ala	Ala	Lys	Ile	Leu	Leu	
	2015					2020						2025				
GAC	CAT	TTT	GCC	AAT	CGA	GAC	ATC	ACA	GAC	CAT	ATG	GAT	CGT	CTT	CCC	6144
Asp	His	Phe	Ala	Asn	Arg	Asp	Ile	Thr	Asp	His	Met	Asp	Arg	Leu	Pro	
					2035						2040				2045	
CGG	GAT	GTG	GCT	CGG	GAT	CGC	ATG	CAC	CAT	GAC	ATT	GTG	CGC	CTT	CTG	6192
Arg	Asp	Val	Ala	Arg	Asp	Arg	Met	His	His	Asp	Ile	Val	Arg	Leu	Leu	
				2050							2055				2060	
GAT	GAA	TAC	AAT	GTG	ACC	CCA	AGC	CCT	CCA	GGC	ACC	GTG	TTG	ACT	TCT	6240
Asp	Glu	Tyr	Asn	Val	Thr	Pro	Ser	Pro	Pro	Gly	Thr	Val	Leu	Thr	Ser	
			2065					2070					2075			
GCT	CTC	TCA	CCT	GTC	ATC	TGT	GGG	CCC	AAC	AGA	TCT	TTC	CTC	AGC	CTG	6288
Ala	Leu	Ser	Pro	Val	Ile	Cys	Gly	Pro	Asn	Arg	Ser	Phe	Leu	Ser	Leu	
		2080					2085					2090				
AAG	CAC	ACC	CCA	ATG	GGC	AAG	AAG	TCT	AGA	CGG	CCC	AGT	GCC	AAG	AGT	6336
Lys	His	Thr	Pro	Met	Gly	Lys	Lys	Ser	Arg	Arg	Pro	Ser	Ala	Lys	Ser	
		2095				2100						2105				
ACC	ATG	CCT	ACT	AGC	CTC	CCT	AAC	CTT	GCC	AAG	GAG	GCA	AAG	GAT	GCC	6384
Thr	Met	Pro	Thr	Ser	Leu	Pro	Asn	Leu	Ala	Lys	Glu	Ala	Lys	Asp	Ala	
	2110				2115						2120				2125	
AAG	GGT	AGT	AGG	AGG	AAG	AAG	TCT	CTG	AGT	GAG	AAG	GTC	CAA	CTG	TCT	6432
Lys	Gly	Ser	Arg	Arg	Lys	Lys	Ser	Leu	Ser	Glu	Lys	Val	Gln	Leu	Ser	
			2130								2135				2140	
GAG	AGT	TCA	GTA	ACT	TTA	TCC	CCT	GTT	GAT	TCC	CTA	GAA	TCT	CCT	CAC	6480
Glu	Ser	Ser	Val	Thr	Leu	Ser	Pro	Val	Asp	Ser	Leu	Glu	Ser	Pro	His	
			2145					2150					2155			
ACG	TAT	GTT	TCC	GAC	ACC	ACA	TCC	TCT	CCA	ATG	ATT	ACA	TCC	CCT	GGG	6528
Thr	Tyr	Val	Ser	Asp	Thr	Thr	Ser	Ser	Pro	Met	Ile	Thr	Ser	Pro	Gly	
		2160					2165					2170				
ATC	TTA	CAG	GCC	TCA	CCC	AAC	CCT	ATG	TTG	GCC	ACT	GCC	GCC	CCT	CCT	6576
Ile	Leu	Gln	Ala	Ser	Pro	Asn	Pro	Met	Leu	Ala	Thr	Ala	Ala	Pro	Pro	
	2175					2180					2185					
GCC	CCA	GTC	CAT	GCC	CAG	CAT	GCA	CTA	TCT	TTT	TCT	AAC	CTT	CAT	GAA	6624
Ala	Pro	Val	His	Ala	Gln	His	Ala	Leu	Ser	Phe	Ser	Asn	Leu	His	Glu	
	2190				2195					2200					2205	
ATG	CAG	CCT	TTG	GCA	CAT	GGG	GCC	AGC	ACT	GTG	CTT	CCC	TCA	GTG	AGC	6672
Met	Gln	Pro	Leu	Ala	His	Gly	Ala	Ser	Thr	Val	Leu	Pro	Ser	Val	Ser	
			2210						2215					2220		
CAG	TTG	CTA	TCC	CAC	CAC	CAC	ATT	GTG	TCT	CCA	GGC	AGT	GGC	AGT	GCT	6720
Gln	Leu	Leu	Ser	His	His	His	Ile	Val	Ser	Pro	Gly	Ser	Gly	Ser	Ala	
			2225					2230					2235			
GGA	AGC	TTG	AGT	AGG	CTC	CAT	CCA	GTC	CCA	GTC	CCA	GCA	GAT	TGG	ATG	6768
Gly	Ser	Leu	Ser	Arg	Leu	His	Pro	Val	Pro	Val	Pro	Ala	Asp	Trp	Met	
		2240					2245					2250				

-144-

AAC CGC ATG GAG GTG AAT GAG ACC CAG TAC AAT GAG ATG TTT GGT ATG Asn Arg Met Glu Val Asn Glu Thr Gln Tyr Asn Glu Met Phe Gly Met 2255 2260 2265	6816
GTC CTG GCT CCA GCT GAG GGC ACC CAT CCT GGC ATA GCT CCC CAG AGC Val Leu Ala Pro Ala Glu Gly Thr His Pro Gly Ile Ala Pro Gln Ser 2270 2275 2280 2285	6864
AGG CCA CCT GAA GGG AAG CAC ATA ACC ACC CCT CGG GAG CCC TTG CCC Arg Pro Pro Glu Gly Lys His Ile Thr Thr Pro Arg Glu Pro Leu Pro 2290 2295 2300	6912
CCC ATT GTG ACT TTC CAG CTC ATC CCT AAA GGC AGT ATT GCC CAA CCA Pro Ile Val Thr Phe Gln Leu Ile Pro Lys Gly Ser Ile Ala Gln Pro 2305 2310 2315	6960
GCG GGG GCT CCC CAG CCT CAG TCC ACC TGC CCT CCA GCT GTT GCG GGC Ala Gly Ala Pro Gln Pro Gln Ser Thr Cys Pro Pro Ala Val Ala Gly 2320 2325 2330	7008
CCC CTG CCC ACC ATG TAC CAG ATT CCA GAA ATG GCC CGT TTG CCC AGT Pro Leu Pro Thr Met Tyr Gln Ile Pro Glu Met Ala Arg Leu Pro Ser 2335 2340 2345	7056
GTG GCT TTC CCC ACT GCC ATG ATG CCC CAG CAG GAC GGG CAG GTA GCT Val Ala Phe Pro Thr Ala Met Met Pro Gln Gln Asp Gly Gln Val Ala 2350 2355 2360 2365	7104
CAG ACC ATT CTC CCA GCC TAT CAT CCT TTC CCA GCC TCT GTG GGC AAG Gln Thr Ile Leu Pro Ala Tyr His Pro Phe Pro Ala Ser Val Gly Lys 2370 2375 2380	7152
TAC CCC ACA CCC CCT TCA CAG CAC AGT TAT GCT TCC TCA AAT GCT GCT Tyr Pro Thr Pro Pro Ser Gln His Ser Tyr Ala Ser Ser Asn Ala Ala 2385 2390 2395	7200
GAG CGA ACA CCC AGT CAC AGT GGT CAC CTC CAG GGT GAG CAT CCC TAC Glu Arg Thr Pro Ser His Ser Gly His Leu Gln Gly Glu His Pro Tyr 2400 2405 2410	7248
CTG ACA CCA TCC CCA GAG TCT CCT GAC CAG TGG TCA AGT TCA TCA CCC Leu Thr Pro Ser Pro Glu Ser Pro Asp Gln Trp Ser Ser Ser Ser Pro 2415 2420 2425	7296
CAC TCT GCT TCT GAC TGG TCA CAT GTG ACC ACC AGC CCT ACC CCT GGG His Ser Ala Ser Asp Trp Ser Asp Val Thr Thr Ser Pro Thr Pro Gly 2430 2435 2440 2445	7344
GGT GCT GGA GGA GGT CAG CGG GGA CCT GGG ACA CAC ATG TCT GAG CCA Gly Ala Gly Gly Gly Gln Arg Gly Pro Gly Thr His Met Ser Glu Pro 2450 2455 2460	7392
CCA CAC AAC AAC ATG CAG GTT TAT GCG TGAGAGAGTC CACCTCCAGT Pro His Asn Asn Met Gln Val Tyr Ala 2465 2470	7439
GTAGAGACAT AACTGACTTT TGTAATGCT GCTGAGGAAC AAATGAAGGT CATCCGGGAG	7499
AGAAATGAAG AAATCTCTGG AGCCAGCTTC TAGAGGTAGG AAAGAGAAGA TGTTCTTATT	7559
CAGATAATGC AAGAGAAGCA ATTCGTCAGT TTCCTGGGT ATCTGCAAGG CTTATTGATT	7619
ATTCTAATCT AATAAGACAA GTTTGTGGAA ATGCAAGATG AATACAAGCC TTGGGTCCAT	7679
GTTTACTCTC TTCTATTTGG AGAATAAGAT GGATGCTTAT TGAAGCCCAG ACATTCTTGC	7739
AGCTTGGACT GCATTTTAAG CCCTGCAGGC TTCTGCCATA TCCATGAGAA GATTCTACAC	7799

TAGCGTCCTG TTGGGAATTA TGCCCTGGAA TTCTGCCTGA ATTGACCTAC GCATCTCCTC	7859
CTCCTTGGAC ATTCTTTTGT CTTCAATTGG TGCTTTTGGT TTTGCACCTC TCCGTGATTG	7919
TAGCCCTACC AGCATGTTAT AGGGCAAGAC CTTTGTGCTT TTGATCATTG TGGCCCATGA	7979
AAGCAACTTT GGTCTCCTTT CCCCTCCTGT CTTCCCGGTA TCCCTTGGAG TCTCACAAGG	8039
TTTACTTTGG TATGGTTCTC AGCACAAACC TTTCAAGTAT GTTGTTCCTT TGGAAAATGG	8099
ACATACTGTA TTGTGTTCTC CTGCATATAT CATTCTGGA GAGAGAAGGG GAGAAGAATA	8159
CTTTTCTTCA ACAAATTTTG GGGGCAGGAG ATCCCTTCAA GAGGCTGCAC CTTAATTTTT	8219
CTTGTCTGTG TGCAGGTCTT CATATAAACT TTACCAGGAA GAAGGGTGTG AGTTTGTGTG	8279
TTTTCTGTGT ATGGGCCTGG TCAGTGTAAG GTTTTATCCT TGATAGTCTA GTTACTATGA	8339
CCCTCCCCAC TTTTTTAAAA CCAGAAAAAG GTTTGGAATG TTGGAATGAC CAAGAGACAA	8399
GTAACTCGT GCAAGAGCCA GTTACCCACC CACAGGTCCC CCTACTTCCT GCCAAGCATT	8459
CCATTGACTG CCTGTATGGA ACACATTTGT CCCAGATCTG AGCATTCTAG GCCTGTTTCA	8519
CTCACTCACC CAGCATATGA AACTAGTCTT AACTGTTGAG CCTTTCCTTT CATATCCACA	8579
GAAGACACTG TCTCAAATGT TGTACCCTTG CCATTTAGGA CTGAACTTTC CTTAGCCCCA	8639
GGGACCCAGT GACAGTTGTC TTCCGTTTGT CAGATGATCA GTCTCTACTG ATTATCTTGC	8699
TGCTTAAAGG CCTGCTCACC AATCTTTCTT TCACACCGTG TGGTCCGTGT TACTGGTATA	8759
CCCAGTATGT TCTCACTGAA GACATGGACT TTATATGTTT AAGTGCAGGA ATTGGAAAGT	8819
TGGACTTGTT TTCTATGATC CAAAACAGCC CTATAAGAAG GTTGGAAAAG GAGGAACTAT	8879
ATAGCAGCCT TTGCTATTTT CTGCTACCAT TTCTTTTCCT CTGAAGCGGC CATGACATTC	8939
CCTTTGGCAA CTAACGTAGA AACTCAACAG AACATTTTCC TTTCCTAGAG TCACCTTTTA	8999
GATGATAATG GACAACTATA GACTTGCTCA TTGTTTCAGAC TGATTGCCCC TCACCTGAAT	9059
CCACTCTCTG TATTCATGCT CTTGGCAATT TCTTTGACTT TCTTTTAAGG GCAGAAGCAT	9119
TTTAGTTAAT TGTAGATAAA GAATAGTTTT CTTCCTCTTC TCCTTGGGCC AGTTAATAAT	9179
TGGTCCATGG CTACACTGCA ACTTCCGTCC AGTGCTGTGA TGCCCATGAC ACCTGCAAAA	9239
TAAGTTCTGC CTGGGCATTT TGTAGATATT AACAGGTGAA TTCCCGACTC TTTTGGTTTG	9299
AATGACAGTT CTCATTCTT CTATGGCTGC AAGTATGCAT CAGTGCTTCC CACTTACCTG	9359
ATTTGTCTGT CGGTGGCCCC ATATGGAAAC CCTGCGTGTC TGTTGGCATA ATAGTTTACA	9419
AATGGTTTTT TCAGTCCTAT CCAAATTTAT TGAACCAACA AAAATAATTA CTTCTGCCCT	9479
GAGATAAGCA GATTAAGTTT GTTCATTCTC TGCTTTATTC TCTCCATGTG GCAACATTCT	9539
GTCAGCCTCT TTCATAGTGT GCAAACATTT TATCATTCTA AATGGTGAAT CTCTGCCCTT	9599
GGACCCATTT ATTATTCACA GATGGGGAGA ACCTATCTGC ATGGACCCTC ACCATCCTCT	9659
GTGCAGCACA CACAGTGCAG GGAGCCAGTG GCGATGGCGA TGACTTTCTT CCCCTGGGAA	9719
TTCC	9723

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a therapeutically effective amount of a Notch protein; and a pharmaceutically acceptable carrier.
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2. The composition of claim 1 in which the Notch protein is a human Notch protein.
3. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising an amino acid sequence encoded by the DNA sequence depicted in Figure 8A (SEQ ID NO:5), 8B (SEQ ID NO:6), 8C (SEQ ID NO:7), 9A (SEQ ID NO:8), or 9B (SEQ ID NO:9), which is able to be bound by an antibody to a Notch protein; and a pharmaceutically acceptable carrier.
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15
4. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising a Notch amino acid sequence depicted in Figure 8A (SEQ ID NO:5), 8B (SEQ ID NO:6), 8C (SEQ ID NO:7), 9A (SEQ ID NO:8), or 9B (SEQ ID NO:9), which displays one or more functional activities associated with a full-length Notch protein; and a pharmaceutically acceptable carrier.
20
5. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising a fragment of a human Notch protein consisting essentially of the extracellular domain of the protein; and a pharmaceutically acceptable carrier.
25
6. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising a region of a Notch protein containing the EGF homologous repeats of the protein; and a pharmaceutically acceptable carrier.
30

7. A pharmaceutical composition comprising a therapeutically effective amount of a fragment of a Notch protein lacking a portion of the EGF-homologous repeats of the protein, which fragment is able to be bound by an antibody to a Notch protein; and a pharmaceutically acceptable carrier.

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8. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising a functionally active portion of a Notch protein; and a pharmaceutically acceptable carrier.

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9. The composition of claim 8 in which the Notch protein is a human Notch protein.

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10. A pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein, said chimeric protein comprising a functionally active portion of a human Notch protein joined via a peptide bond to a sequence of a protein different from the Notch protein; and a pharmaceutically acceptable carrier.

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11. The composition of claim 10 in which the functionally active portion of the Notch protein is encoded by the human cDNA sequence contained in plasmid hN3k as deposited with the ATCC and assigned accession number 68609, or encoded by the human cDNA sequence contained in plasmid hN5k as deposited with the ATCC and assigned accession number 68611.

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12. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising the amino acid sequence depicted in Figure 10 (SEQ ID NO:11); and a pharmaceutically acceptable carrier.

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13. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising the amino acid sequence

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depicted in Figure 11 (SEQ ID NO:13); and a pharmaceutically acceptable carrier.

14. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising the portion of a human Notch protein with the greatest homology to the epidermal growth factor-like repeats 11 and 12 of the *Drosophila* Notch sequence as shown in Figure 4 (SEQ ID NO:14); and a pharmaceutically acceptable carrier.

15. A pharmaceutical composition comprising a therapeutically effective amount of a derivative or analog of a Notch protein, which derivative or analog is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Delta protein expressed on the surface of a second cell; and a pharmaceutically acceptable carrier.

16. A pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein, said chimeric protein comprising a Notch protein joined via a peptide bond to a protein sequence of a protein different from the Notch protein; and a pharmaceutically acceptable carrier.

17. A pharmaceutical composition comprising a therapeutically effective amount of a fragment of a Notch protein, which fragment is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Delta protein expressed on the surface of a second cell; and a pharmaceutically acceptable carrier.

18. A pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein, said chimeric protein comprising a fragment of a Notch protein joined via a peptide bond to a protein sequence of a protein different from the Notch protein, which fragment is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Delta

protein expressed on the surface of a second cell; and a pharmaceutically acceptable carrier.

19. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising a derivative or analog of a Delta protein, which derivative or analog is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell; and a pharmaceutically acceptable carrier.

20. A pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein, said chimeric protein comprising a fragment of a Delta protein joined via a peptide bond to a protein sequence of a protein different from the Delta protein, which fragment is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell; and a pharmaceutically acceptable carrier.

21. A pharmaceutical composition comprising a therapeutically effective amount of a protein, said protein comprising a derivative or analog of a Serrate protein, which derivative or analog is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell; and a pharmaceutically acceptable carrier.

22. A pharmaceutical composition comprising a therapeutically effective amount of a derivative or analog of a Notch protein, which derivative or analog is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a second protein expressed on the surface of a second cell, which second protein is selected from the group consisting of a Delta protein and a Serrate protein; and a pharmaceutically acceptable carrier.

23. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a Notch protein; and a pharmaceutically acceptable carrier.

5 24. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a functionally active portion of a human Notch protein; and a pharmaceutically acceptable carrier.

25. A pharmaceutical composition comprising a therapeutically
10 effective amount of a nucleic acid encoding the amino acid sequence depicted in Figure 10 (SEQ ID NO:11); and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding the amino acid sequence depicted in
15 Figure 11 (SEQ ID NO:13); and a pharmaceutically acceptable carrier.

27. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a fragment of a Notch protein, which fragment is characterized by the ability *in vitro*, when expressed on the surface of
20 a first cell, to bind to a Delta protein expressed on the surface of a second cell; and a pharmaceutically acceptable carrier.

28. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a chimeric protein, said chimeric
25 protein comprising a functionally active fragment of a human Notch protein joined via a peptide bond to a protein sequence of a protein different from the Notch protein; and a pharmaceutically acceptable carrier.

29. A pharmaceutical composition comprising a therapeutically
30 effective amount of a nucleic acid encoding a fragment of a Delta protein, which fragment is characterized by the ability *in vitro*, when expressed on the surface of

a first cell, to bind to a Notch protein expressed on the surface of a second cell; and a pharmaceutically acceptable carrier.

30. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a fragment of a Serrate protein, which fragment is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell; and a pharmaceutically acceptable carrier.

31. The composition of claim 24 in which the nucleic acid is a nucleic acid vector.

32. A pharmaceutical composition comprising a therapeutically effective amount of an antibody which binds to a Notch protein; and a pharmaceutically acceptable carrier.

33. A pharmaceutical composition comprising a therapeutically effective amount of a fragment or derivative of an antibody to a Notch protein containing the idiotype of the antibody; and a pharmaceutically acceptable carrier.

34. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of a molecule which antagonizes the function of a Notch protein.

35. The method according to claim 34 in which the disease or disorder is a malignancy characterized by increased Notch activity or increased expression of a Notch protein or of a Notch derivative capable of being bound by an anti-Notch antibody, relative to said Notch activity or expression in an analogous non-malignant sample.

36. The method according to claim 34 in which the disease or disorder is cervical cancer.

5 37. The method according to claim 34 in which the disease or disorder is breast cancer.

38. The method according to claim 34 in which the disease or disorder is colon cancer.

10 39. The method according to claim 35 in which the malignancy is selected from the group consisting of melanoma, seminoma, and lung cancer.

40. The method according to claim 35 in which the subject is a human.
15

41. The method according to claim 36, 37 or 38 in which the molecule is an antibody to Notch or a portion of said antibody containing the binding domain thereof.

20 42. The method according to claim 36, 37 or 38 in which the molecule is a protein consisting of at least the extracellular domain of a Notch protein or a portion thereof capable of binding to a Notch ligand.

25 43. The method according to claim 36, 37 or 38 in which the molecule is a protein consisting of at least the EGF homologous repeats of a Notch protein.

30 44. The method according to claim 36, 37 or 38 in which the molecule is a protein consisting of at least an adhesive fragment of a Notch protein.

45. The method according to claim 36, 37 or 38 in which the molecule is an oligonucleotide which (a) consists of at least six nucleotides; (b) comprises a sequence complementary to at least a portion of an RNA transcript of a Notch gene; and (c) is hybridizable to the RNA transcript.

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46. A method of treating or preventing a disease or disorder in a subject in need of such treatment or prevention comprising administering to the subject a therapeutically effective amount of a molecule which promotes the function of a Notch protein.

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47. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a Notch protein.

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48. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a functionally active portion of a Notch protein.

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49. The method according to claim 47 in which the Notch protein is a human Notch protein.

25

50. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a chimeric protein, said protein comprising a functionally active portion of a Notch protein joined via a peptide bond to a protein sequence of a protein different from the Notch protein.

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51. The method according to claim 49 in which the human Notch protein comprises the amino acid sequence depicted in Figure 10 (SEQ ID NO:11) or Figure 11 (SEQ ID NO:13).

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52. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a derivative or analog of a Notch protein, which derivative or analog is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a second protein expressed on the surface of a second cell, which second protein is selected from the group consisting of a Delta protein and a Serrate protein.

53. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a derivative or analog of a Delta protein, which derivative or analog is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell.

54. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a derivative or analog of a Serrate protein, which derivative or analog is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell.

55. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a nucleic acid encoding a Notch protein.

56. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a nucleic acid encoding a functionally active portion of a Notch protein.

57. The method according to claim 55 in which the subject is human and the Notch protein is a human Notch protein.

58. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a nucleic acid encoding a fragment of a Notch protein, which fragment is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a second protein expressed on the surface of a second cell, which second protein is selected from the group consisting of a Delta protein and a Serrate protein.

59. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a nucleic acid encoding a fragment of a Delta protein, which fragment is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell.

60. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a nucleic acid encoding a fragment of a Serrate protein, which fragment is characterized by the ability *in vitro*, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell.

61. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of antibody to a Notch protein.

62. The method according to claim 58 in which the antibody is monoclonal.

63. A method for treating a patient with a tumor, of a tumor type characterized by expression of a Notch gene, comprising administering to the patient an effective amount of an oligonucleotide, which oligonucleotide (a) consists of at least six nucleotides; (b) comprises a sequence complementary to at

least a portion of an RNA transcript of the Notch gene; and (c) is hybridizable to the RNA transcript.

5 64. The method according to claim 60 in which the patient is a human, and the Notch gene is a human gene.

65. An isolated oligonucleotide consisting of at least six nucleotides, and comprising a sequence complementary to at least a portion of an RNA transcript of a Notch gene, which oligonucleotide is hybridizable to the
10 RNA transcript.

66. A pharmaceutical composition comprising the oligonucleotide of claim 65; and a pharmaceutically acceptable carrier.

15 67. A method of inhibiting the expression of a nucleic acid sequence encoding a Notch protein in a cell comprising providing the cell with an effective amount of the oligonucleotide of claim 65.

20 68. A method of diagnosing a disease or disorder characterized by an aberrant level of Notch protein or activity in a patient, comprising measuring the level of Notch protein expression or activity in a sample derived from the patient, in which an increase or decrease in Notch protein or activity in the patient sample relative to the level found in such a sample from a normal individual indicates the presence of the disease or disorder in the patient.

25 69. A method of diagnosing a malignancy characterized by an increased amount of a Notch protein or of a Notch derivative capable of being bound by an anti-Notch antibody, comprising measuring the amount of a Notch protein or of a Notch derivative capable of being bound by an anti-Notch
30 antibody, in a sample containing or suspected of containing malignant cells from a patient, in which an increase in the amount of the Notch protein or of the Notch

derivative capable of being bound by an anti-Notch antibody, in the sample, relative to said amount found in an analogous sample of non-malignant cells indicates the presence of the disease or disorder in the patient.

5 70. The method according to claim 69 in which the malignancy is cervical cancer.

71. The method according to claim 69 in which the malignancy is breast cancer.

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72. The method according to claim 69 in which the malignancy is colon cancer.

15 73. The method according to claim 69 in which the malignancy is selected from the group consisting of melanoma, seminoma, and lung cancer.

74. The method according to claim 69 in which the amount of the Notch protein or derivative is measured by a method comprising contacting the sample with an anti-Notch antibody such that immunospecific binding can occur, and measuring the amount of any immunospecific binding of the antibody that occurs.

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75. A method of treating or preventing a nervous system disorder in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a functionally active portion of a Notch protein.

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76. A method of promoting tissue regeneration or repair in a subject comprising administering to a subject an effective amount of a functionally active portion of a Notch protein.

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77. A method of treating a benign dysproliferative disorder in a subject comprising administering to a subject in need of such treatment an effective amount of a functionally active portion of a Notch protein, in which the disorder is selected from the group consisting of cirrhosis of the liver, psoriasis, keloids, and baldness.

78. A substantially purified human Notch protein comprising the amino acid sequence encoded by the hN homolog as depicted in Figure 13 from amino acid numbers 1 through 2169 (SEQ ID NO:19).

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79. A substantially purified human Notch protein comprising the amino acid sequence encoded by the hN homolog as depicted in Figure 13 from amino acid numbers about 26 through 2169 (as contained in SEQ ID NO:19).

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80. A substantially purified protein comprising the extracellular domain of the mature human Notch protein encoded by the hN homolog, as depicted in Figure 13 from amino acid numbers about 26 through 1677 (as contained in SEQ ID NO:19).

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81. A substantially purified protein comprising the EGF homologous repeats of the mature human Notch protein encoded by the hN homolog, as depicted in Figure 13 from amino acid numbers 26 through 1413 (as contained in SEQ ID NO:19).

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82. A substantially purified protein comprising the EGF like repeats 11 and 12 of the mature human Notch protein encoded by the hN homolog, as depicted in Figure 13 (as contained in SEQ ID NO:19).

83. A substantially purified protein consisting essentially of the extracellular domain of the mature human Notch protein encoded by the hN

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homolog, as depicted in Figure 13 from amino acid numbers about 26 through 1677 (as contained in SEQ ID NO:19).

5 84. A substantially purified nucleic acid encoding the protein of claim 78.

85. A substantially purified nucleic acid encoding the protein of claim 79.

10 86. A substantially purified nucleic acid encoding the protein of claim 80.

15 87. A substantially purified nucleic acid encoding the protein of claim 82.

88. The nucleic acid of claim 85 which is a DNA molecule comprising the sequence depicted in Figure 17 from nucleotide numbers 82 through 7419 (as contained in SEQ ID NO:21).

20 89. The nucleic acid of claim 80 in which the sequence encoding the extracellular domain is as presented in Figure 17 (as contained in SEQ ID NO:21).

25 90. A recombinant cell containing the nucleic acid of claim 84, 87 or 88.

30 91. The composition of claim 2 in which the Notch protein comprises the amino acid sequence encoded by the hN homolog as depicted in Figure 13 from amino acid numbers 26 through 2169 (as contained in SEQ ID NO:19).

92. A composition comprising a therapeutically effective amount of a Notch protein or Notch derivative, said derivative being capable of being bound by an anti-Notch antibody, for use as a medicament.

5 93. A composition comprising a therapeutically effective amount of a molecule which antagonizes the function of a Notch protein, for use as a medicament.

10 94. Use of a composition comprising a molecule which antagonizes the function of a Notch protein, for the manufacture of a medicament for the treatment of cervical cancer, breast cancer, or colon cancer.

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1/68

GAATTCGGAG GAATTATTCA AAACATAAAC ACAATAAACA ATTTGAGTAG TTGCCGCACA	60
CACACACACA CACAGCCCGT GGATTATTAC ACTAAAAGCG ACACTCAATC CAAAAAATCA	120
GCAACAAAAA CATCAATAAA C ATG CAT TGG ATT AAA TGT TTA TTA ACA GCA	171
Met His Trp Ile Lys Cys Leu Leu Thr Ala	
1 5 10	
TTC ATT TGC TTC ACA GTC ATC GTG CAG GTT CAC AGT TCC GGC AGC TTT	219
Phe Ile Cys Phe Thr Val Ile Val Gln Val His Ser Ser Gly Ser Phe	
15 20 25	
GAG TTG CGC CTG AAG TAC TTC AGC AAC GAT CAC GGG CGG GAC AAC GAG	267
Glu Leu Arg Leu Lys Tyr Phe Ser Asn Asp His Gly Arg Asp Asn Glu	
30 35 40	
GGT CGC TGC TGC AGC GGG GAG TCG GAC GGA GCG ACG GGC AAG TGC CTG	315
Gly Arg Cys Cys Ser Gly Glu Ser Asp Gly Ala Thr Gly Lys Cys Leu	
45 50 55	
GGC AGC TGC AAG ACG CGG TTT CGC GTC TGC CTA AAG CAC TAC CAG GCC	363
Gly Ser Cys Lys Thr Arg Phe Arg Val Cys Leu Lys His Tyr Gln Ala	
60 65 70	
ACC ATC GAC ACC ACC TCC CAG TGC ACC TAC GGG GAC GTG ATC ACG CCC	411
Thr Ile Asp Thr Thr Ser Gln Cys Thr Tyr Gly Asp Val Ile Thr Pro	
75 80 85 90	
ATT CTC GGC GAG AAC TCG GTC AAT CTG ACC GAC GCC CAG CGC TTC CAG	459
Ile Leu Gly Glu Asn Ser Val Asn Leu Thr Asp Ala Gln Arg Phe Gln	
95 100 105	
AAC AAG GGC TTC ACG AAT CCC ATC CAG TTC CCC TTC TCG TTC TCA TGG	507
Asn Lys Gly Phe Thr Asn Pro Ile Gln Phe Pro Phe Ser Phe Ser Trp	
110 115 120	

FIG.1A

2/68

CCG GGT ACC TTC TCG CTG ATC GTC GAG GCC TGG CAT GAT ACG AAC AAT Pro Gly Thr Phe Ser Leu Ile Val Glu Ala Trp His Asp Thr Asn Asn 125 130 135	555
AGC GGC AAT GCG CGA ACC AAC AAG CTC CTC ATC CAG CGA CTC TTG GTG Ser Gly Asn Ala Arg Thr Asn Lys Leu Leu Ile Gln Arg Leu Leu Val 140 145 150	603
CAG CAG GTA CTG GAG GTG TCC TCC GAA TGG AAG ACG AAC AAG TCG GAA Gln Gln Val Leu Glu Val Ser Ser Glu Trp Lys Thr Asn Lys Ser Glu 155 160 165 170	651
TCG CAG TAC ACG TCG CTG GAG TAC GAT TTC CGT GTC ACC TGC GAT CTC Ser Gln Tyr Thr Ser Leu Glu Tyr Asp Phe Arg Val Thr Cys Asp Leu 175 180 185	699
AAC TAC TAC GGA TCC GGC TGT GCC AAG TTC TGC CGG CCC CGC GAC GAT Asn Tyr Tyr Gly Ser Gly Cys Ala Lys Phe Cys Arg Pro Arg Asp Asp 190 195 200	747
TCA TTT GGA CAC TCG ACT TGC TCG GAG ACG GGC GAA ATT ATC TGT TTG Ser Phe Gly His Ser Thr Cys Ser Glu Thr Gly Glu Ile Ile Cys Leu 205 210 215	795
ACC GGA TGG CAG GGC GAT TAC TGT CAC ATA CCC AAA TGC GCC AAA GGC Thr Gly Trp Gln Gly Asp Tyr Cys His Ile Pro Lys Cys Ala Lys Gly 220 225 230	843
TGT GAA CAT GGA CAT TGC GAC AAA CCC AAT CAA TGC GTT TGC CAA CTG Cys Glu His Gly His Cys Asp Lys Pro Asn Gln Cys Val Cys Gln Leu 235 240 245 250	891
GGC TGG AAG GGA GCC TTG TGC AAC GAG TGC GTT CTG GAA CCG AAC TGC Gly Trp Lys Gly Ala Leu Cys Asn Glu Cys Val Leu Glu Pro Asn Cys 255 260 265	939

FIG.1B

3/68

ATC CAT GGC ACC TGC AAC AAA CCC TGG ACT TGC ATC TGC AAC GAG GGT	987
Ile His Gly Thr Cys Asn Lys Pro Trp Thr Cys Ile Cys Asn Glu Gly	
270 275 280	
TGG GGA GGC TTG TAC TGC AAC CAG GAT CTG AAC TAC TGC ACC AAC CAC	1035
Trp Gly Gly Leu Tyr Cys Asn Gln Asp Leu Asn Tyr Cys Thr Asn His	
285 290 295	
AGA CCC TGC AAG AAT GGC GGA ACC TGC TTC AAC ACC GGC GAG GGA TTG	1083
Arg Pro Cys Lys Asn Gly Gly Thr Cys Phe Asn Thr Gly Glu Gly Leu	
300 305 310	
TAC ACA TGC AAA TGC GCT CCA GGA TAC AGT GGT GAT GAT TGC GAA AAT	1131
Tyr Thr Cys Lys Cys Ala Pro Gly Tyr Ser Gly Asp Asp Cys Glu Asn	
315 320 325 330	
GAG ATC TAC TCC TGC GAT GCC GAT GTC AAT CCC TGC CAG AAT GGT GGT	1179
Glu Ile Tyr Ser Cys Asp Ala Asp Val Asn Pro Cys Gln Asn Gly Gly	
335 340 345	
ACC TGC ATC GAT GAG CCG CAC ACA AAA ACC GGC TAC AAG TGT CAT TGC	1227
Thr Cys Ile Asp Glu Pro His Thr Lys Thr Gly Tyr Lys Cys His Cys	
350 355 360	
GCC AAC GGC TGG AGC GGA AAG ATG TGC GAG GAG AAA GTG CTC ACG TGT	1275
Ala Asn Gly Trp Ser Gly Lys Met Cys Glu Glu Lys Val Leu Thr Cys	
365 370 375	
TCG GAC AAA CCC TGT CAT CAG GGA ATC TGC CGC AAC GTT CGT CCT GGC	1323
Ser Asp Lys Pro Cys His Gln Gly Ile Cys Arg Asn Val Arg Pro Gly	
380 385 390	
TTG GGA AGC AAG GGT CAG GGC TAC CAG TGC GAA TGT CCC ATT GGC TAC	1371
Leu Gly Ser Lys Gly Gln Gly Tyr Gln Cys Glu Cys Pro Ile Gly Tyr	
395 400 405 410	

FIG.1C

4/68

AGC GGA CCC AAC TGC GAT CTC CAG CTG GAC AAC TGC AGT CCG AAT CCA Ser Gly Pro Asn Cys Asp Leu Gln Leu Asp Asn Cys Ser Pro Asn Pro 415 420 425	1419
TGC ATA AAC GGT GGA AGC TGT CAG CCG AGC GGA AAG TGT ATT TGC CCA Cys Ile Asn Gly Gly Ser Cys Gln Pro Ser Gly Lys Cys Ile Cys Pro 430 435 440	1467
GCG GGA TTT TCG GGA ACG AGA TGC GAG ACC AAC ATT GAC GAT TGT CTT Ala Gly Phe Ser Gly Thr Arg Cys Glu Thr Asn Ile Asp Asp Cys Leu 445 450 455	1515
GGC CAC CAG TGC GAG AAC GGA GGC ACC TGC ATA GAT ATG GTC AAC CAA Gly His Gln Cys Glu Asn Gly Gly Thr Cys Ile Asp Met Val Asn Gln 460 465 470	1563
TAT CGC TGC CAA TGC GTT CCC GGT TTC CAT GGC ACC CAC TGT AGT AGC Tyr Arg Cys Gln Cys Val Pro Gly Phe His Gly Thr His Cys Ser Ser 475 480 485 490	1611
AAA GTT GAC TTG TGC CTC ATC AGA CCG TGT GCC AAT GGA GGA ACC TGC Lys Val Asp Leu Cys Leu Ile Arg Pro Cys Ala Asn Gly Gly Thr Cys 495 500 505	1659
TTG AAT CTC AAC AAC GAT TAC CAG TGC ACC TGT CGT GCG GGA TTT ACT Leu Asn Leu Asn Asn Asp Tyr Gln Cys Thr Cys Arg Ala Gly Phe Thr 510 515 520	1707
GGC AAG GAT TGC TCT GTG GAC ATC GAT GAG TGC AGC AGT GGA CCC TGT Gly Lys Asp Cys Ser Val Asp Ile Asp Glu Cys Ser Ser Gly Pro Cys 525 530 535	1755
CAT AAC GGC GGC ACT TGC ATG AAC CGC GTC AAT TCG TTC GAA TGC GTG His Asn Gly Gly Thr Cys Met Asn Arg Val Asn Ser Phe Glu Cys Val 540 545 550	1803

FIG.1D

5/68

TGT GCC AAT GGT TTC AGG GGC AAG CAG TGC GAT GAG GAG TCC TAC GAT Cys Ala Asn Gly Phe Arg Gly Lys Gln Cys Asp Glu Glu Ser Tyr Asp 555 560 565 570	1851
TCG GTG ACC TTC GAT GCC CAC CAA TAT GGA GCG ACC ACA CAA GCG AGA Ser Val Thr Phe Asp Ala His Gln Tyr Gly Ala Thr Thr Gln Ala Arg 575 580 585	1899
GCC GAT GGT TTG ACC AAT GCC CAG GTA GTC CTA ATT GCT GTT TTC TCC Ala Asp Gly Leu Thr Asn Ala Gln Val Val Leu Ile Ala Val Phe Ser 590 595 600	1947
GTT GCG ATG CCT TTG GTG GCG GTT ATT GCG GCG TGC GTG GTC TTC TGC Val Ala Met Pro Leu Val Ala Val Ile Ala Ala Cys Val Val Phe Cys 605 610 615	1995
ATG AAG CGC AAG CGT AAG CGT GCT CAG GAA AAG GAC GAC GCG GAG GCC Met Lys Arg Lys Arg Lys Arg Ala Gln Glu Lys Asp Asp Ala Glu Ala 620 625 630	2043
AGG AAG CAG AAC GAA CAG AAT GCG GTG GCC ACA ATG CAT CAC AAT GGC Arg Lys Gln Asn Glu Gln Asn Ala Val Ala Thr Met His His Asn Gly 635 640 645 650	2091
AGT GGG GTG GGT GTA GCT TTG GCT TCA GCC TCT CTG GGC GGC AAA ACT Ser Gly Val Gly Val Ala Leu Ala Ser Ala Ser Leu Gly Gly Lys Thr 655 660 665	2139
GGC AGC AAC AGC GGT CTC ACC TTC GAT GGC GGC AAC CCG AAT ATC ATC Gly Ser Asn Ser Gly Leu Thr Phe Asp Gly Gly Asn Pro Asn Ile Ile 670 675 680	2187
AAA AAC ACC TGG GAC AAG TCG GTC AAC AAC ATT TGT GCC TCA GCA GCA Lys Asn Thr Trp Asp Lys Ser Val Asn Asn Ile Cys Ala Ser Ala Ala 685 690 695	2235

FIG.1E

6/68

GCA GCG GCG GCG GCG GCA GCA GCG GCG GAC GAG TGT CTC ATG TAC GGC Ala Ala Ala Ala Ala Ala Ala Ala Ala Asp Glu Cys Leu Met Tyr Gly 700 705 710	2283
GGA TAT GTG GCC TCG GTG GCG GAT AAC AAC AAT GCC AAC TCA GAC TTT Gly Tyr Val Ala Ser Val Ala Asp Asn Asn Asn Ala Asn Ser Asp Phe 715 720 725 730	2331
TGT GTG GCT CCG CTA CAA AGA GCC AAG TCG CAA AAG CAA CTC AAC ACC Cys Val Ala Pro Leu Gln Arg Ala Lys Ser Gln Lys Gln Leu Asn Thr 735 740 745	2379
GAT CCC ACG CTC ATG CAC CGC GGT TCG CCG GCA GGC AGC TCA GCC AAG Asp Pro Thr Leu Met His Arg Gly Ser Pro Ala Gly Ser Ser Ala Lys 750 755 760	2427
GGA GCG TCT GGC GGA GGA CCG GGA GCG GCG GAG GGC AAG AGG ATC TCT Gly Ala Ser Gly Gly Gly Pro Gly Ala Ala Glu Gly Lys Arg Ile Ser 765 770 775	2475
GTT TTA GGC GAG GGT TCC TAC TGT AGC CAG CGT TGG CCC TCG TTG GCG Val Leu Gly Glu Gly Ser Tyr Cys Ser Gln Arg Trp Pro Ser Leu Ala 780 785 790	2523
GCG GCG GGA GTG GCC GGA GCC TGT TCA TCC CAG CTA ATG GCT GCA GCT Ala Ala Gly Val Ala Gly Ala Cys Ser Ser Gln Leu Met Ala Ala Ala 795 800 805 810	2571
TCG GCA GCG GGC AGC GGA GCG GGG ACG GCG CAA CAG CAG CGA TCC GTG Ser Ala Ala Gly Ser Gly Ala Gly Thr Ala Gln Gln Gln Arg Ser Val 815 820 825	2619
GTC TGC GGC ACT CCG CAT ATG TAACTCCAAA AATCCGGAAG GGCTCCTGGT Val Cys Gly Thr Pro His Met 830	2670
AAATCCGGAG AAATCCGCAT GGAGGAGCTG ACAGCACATA CACAAAGAAA AGACTGGGTT GGGTTCAAAA TGTGAGAGAG ACGCCAAAAT GTTGTGTGTG ATTGAAGCAG TTTAGTCGTC ACGAAAAATG AAAAATCTGT AACAGGCATA ACTCGTAAAC TCCCTAAAAA ATTTGTATAG TAATTAGCAA AGCTGTGACC CAGCCGTTTC GATCCCGAAT TC	2730 2790 2850 2892

FIG.1F

SUBSTITUTE SHEET (RULE 26)

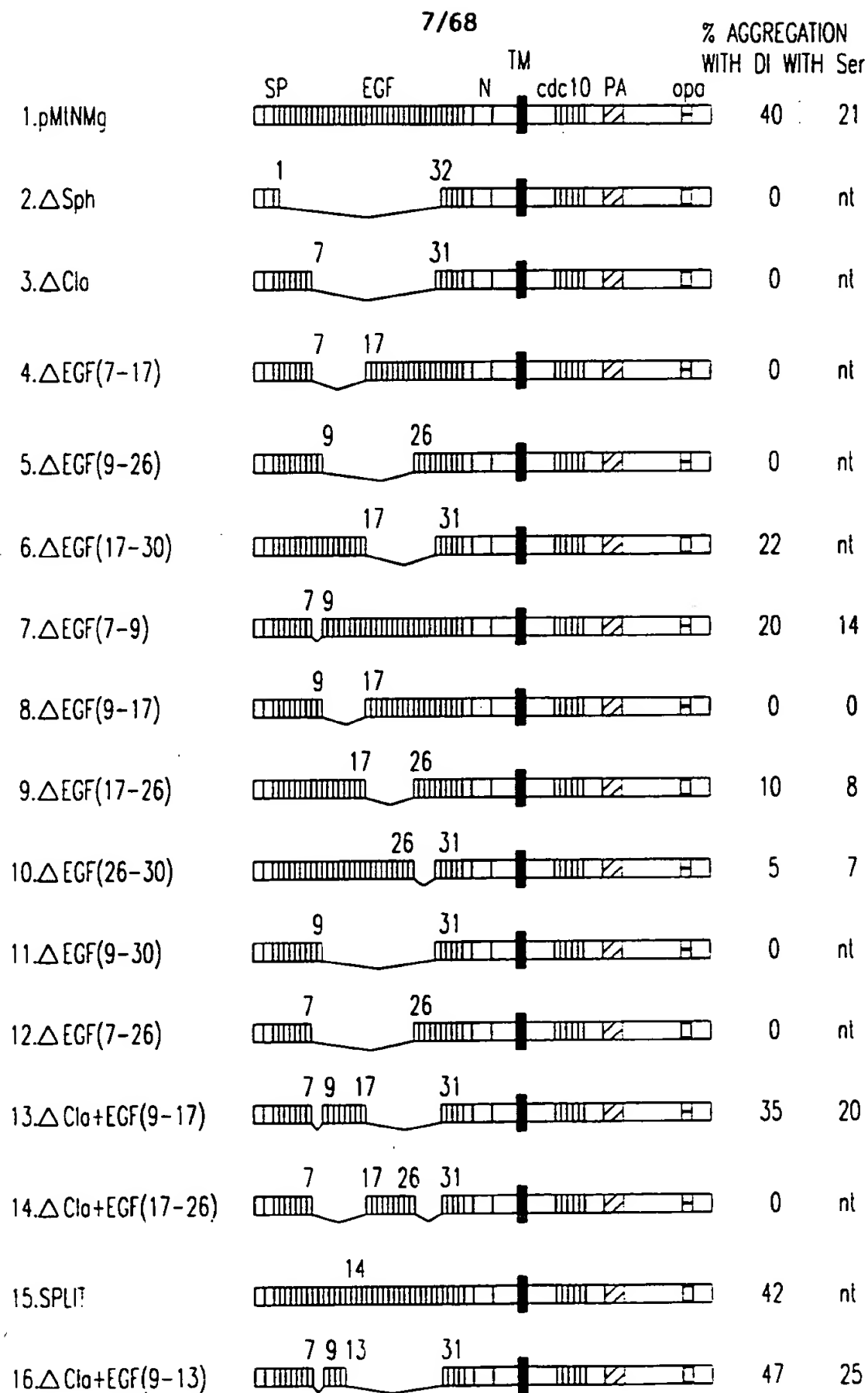


FIG.2A

8/68

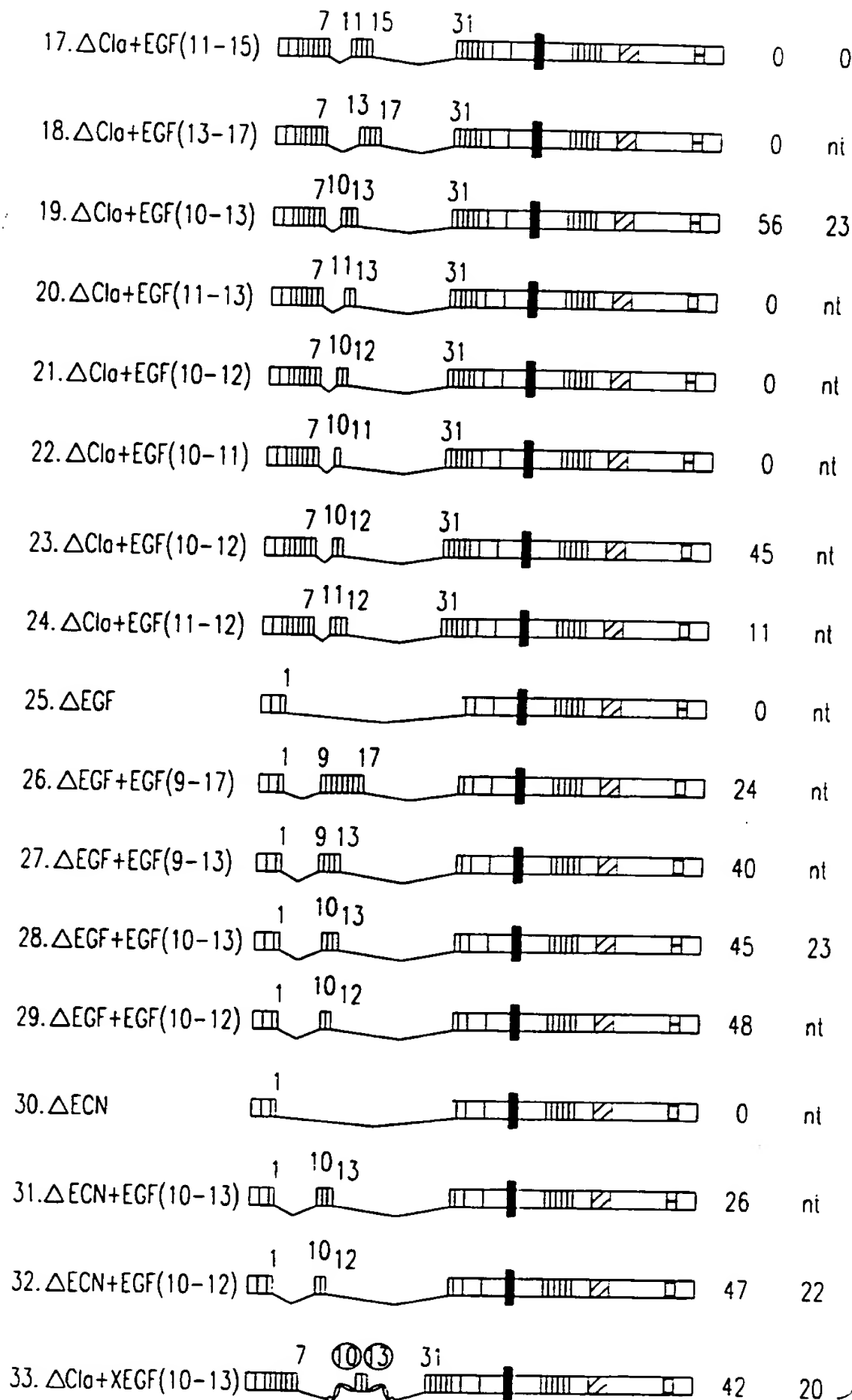


FIG.2B

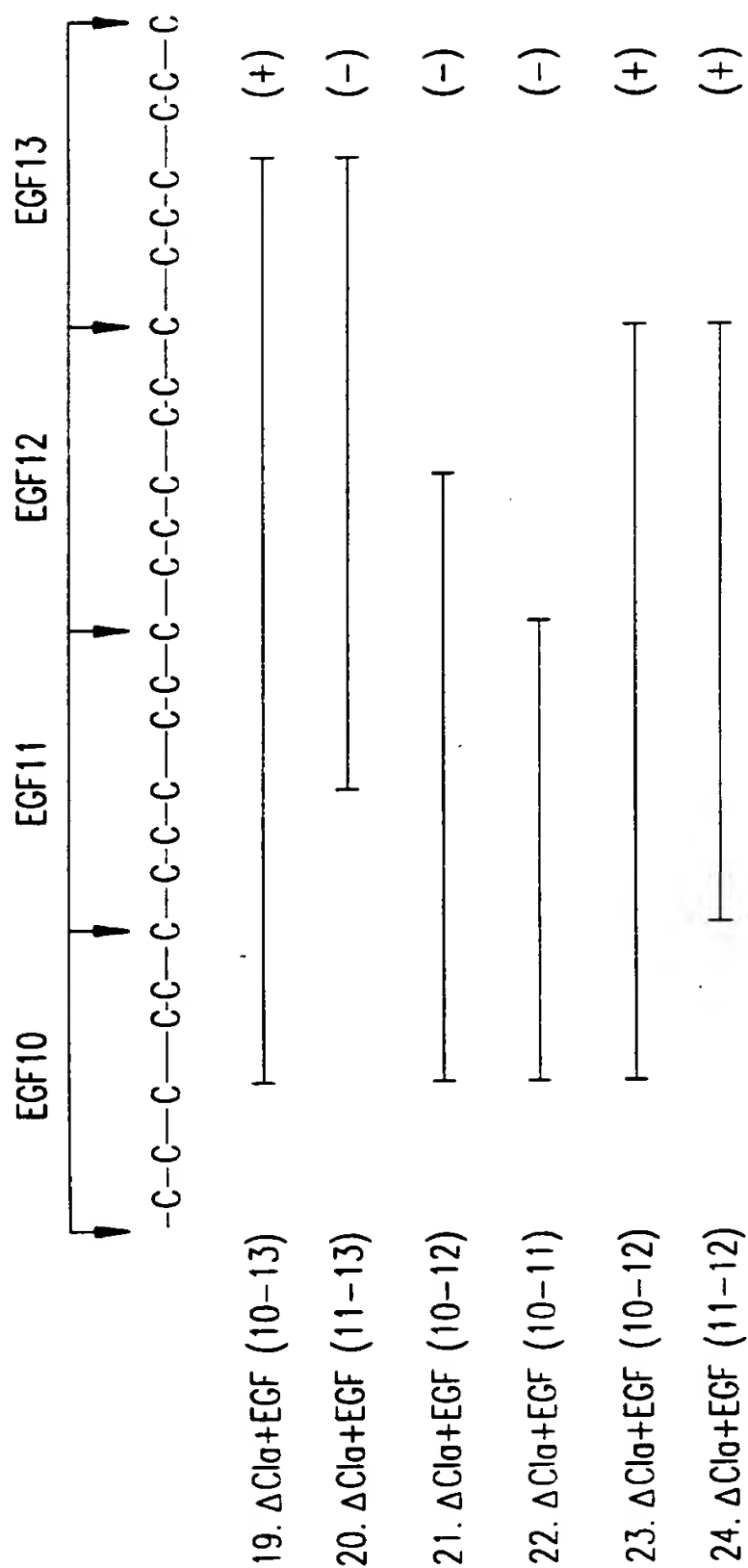


FIG. 3

10/68

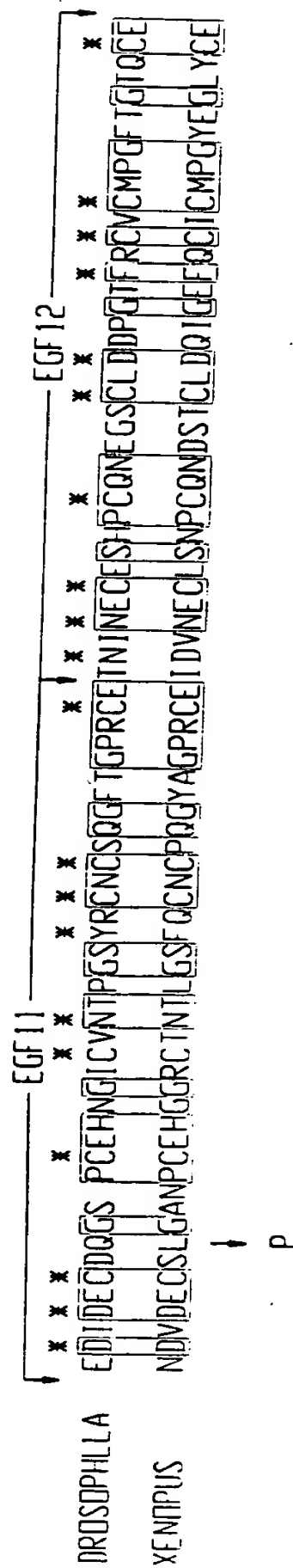


FIG.4

11/68

FIG. 5A

1 CCGAGTCGAGCGCGGTGCTTCGAGCGGTGATGAGCCCCCTTCTGTCAACGCTAAAGATC
 121 AAGCACATACIAAGGTCCATAATAATAATAATAATATGTTGTGTGATACCAACATATAT
 241 GGGCGTTATTCAGCTATCCAGAGCAAGTGTAGTGTGGCAAAATAGAAACAAACAAAGGCA
 361 CAATCCAGAGTGAATCCGAAACAACCTCCATCTAGATCGCCCAACCAGCATCAGCTCGCA

 481 TCGTCGTGGAGTCAACAATAGAAATCAGCAGACAGCCTGGGAATGTCCAAGAGACGGCG
 SerSerLeuGluSerThrIleGluSerAlaAspSerLeuGlyMetSerLysLysThrAla

 601 CCGGATGTGCGATCATTAAGTCTGCCCTGCAACTTAATTCCTTTAATTTAATACIGTTA
 ArgAspCysArgSerLeuLysSerAlaCysAsnLeuIleAlaLeuIleLeuLeu

 721 AACAGCCATCTACTCAACGGCTATTCGTCGGGATGCCAGCGGAACCTAGGGCCACCAAG
 AsnSerHisLeuLeuAsnGlyTyrCysCysGlyMetProAlaGluLeuArgAlaThrLys

 841 ACCGAGCAGGGTCCAGCATATCCACGGGTGTTCGTTTGGCAAGGCCACCACCAAGATA
 ThrGluGlnGlyAlaSerIleSerThrGlyCysSerPheGlyAsnAlaThrThrLysIle
 #2
 961 ACGTTTCGTGGACGAAGTCGTTACGCTGATACTCAGGCGTGGATATGTACACACACA
 ThrPheArgIrpThrLysSerPheThrLeuIleLeuGlnAlaLeuAspMetTyrAsnThr
 #3
 1081 TCGCCGGAGTGGAGACCCCTGGACCACATCGGGCGGAACGGCGGATCACCTACCGTGTC
 SerProGluIrpLysThrLeuAspHisIleGlyArgAsnAlaArgIleThrTyrArgVal

 1201 GACGATCAGTTCGGTCACTACGCCCTCGGCTCCGAGGGTCAGAAAGCTTCGCCIGAAATGGC
 AspAspGlnPheGlyHisTyrAlaCysGlySerGluGlyGlnLysLeuCysLeuAsnGly

12/68

TACAAACATCAGCGCCTATCAAGTGGAGGTCAAGTGTGACAAACAAAAACGAGAG
 CCAACAAACCAACAAACGAGGCAAGTGGAGAAATGATACAGCATCCAGAGTAC
 CCAAAATCTGCATACATGGCTAATTAGGCTGCCAGCGAATTACATTGTGGTGC
 AACGCCCCAGAAATGTACAAAATGTTAGGAAACATTTCCGGGAAACAGCTACGTCG
 MetPheArgLysHisPheArgArgLysProAlaThrSer 13
 ACAAAAGGCAGCGTCCGAGGCATCGGTACCCAAAATCGCGACCCCTGCCATCGACGATC
 ThrLysArgGlnArgProArgHisArgValProLysIleAlaThrLeuProSerThrIle 53
 GTCATAGGATATCCGCAGCTGGTAACCTCGAGCTGGAAATATAGAAATCTCAAAATACC
 ValHisLysIleSerAlaAlaGlyAsnPheGluLeuGluIleLeuGluIleSerAsnThr 93

 #1
 ACGATAGGCTGCTCGCCATGCACGACGGCATTCGGGCTGTGCTGAGGAGTACCAGACC
 ThrIleGlyCysSerProCysThrThrAlaPheArgLeuCysLeuLysGluTyrGlnThr 133
 CTGGGTGGCTCCAGGTTTGTGCTCAGCGATCCGGGTGTGGGAGCCATTGTGTGCCCTTT
 LeuGlyGlySerSerPheValLeuSerAspProGlyValGlyAlaIleValLeuProPhe 173
 TCCTATCCAGATCGGGAGAGGTAAATTGAGGAACATCATCTCGGGGTGATACGCGG
 SerTyrProAspAlaGluArgLeuIleGluGluThrSerTyrSerGlyValIleLeuPro 213
 CCGGTGCAATGGCGCGTTACCTACTACAACACGACCTGCACGACCTTGTCCGTCGCGGG
 ArgValGlnCysAlaValThrTyrTyrAsnThrThrCysThrThrPheCysArgProArg 253
 #4
 TGGCAGGGCGTCAACTGCGAGGAGGCCATATGCCAAGCGGGCTGCGACCCCGTCCACGGC
 TrpGlnGlyValAsnCysGluGluAlaIleCysLysAlaGlyCysAspProValHisGly 293

FIG.5B

13/68

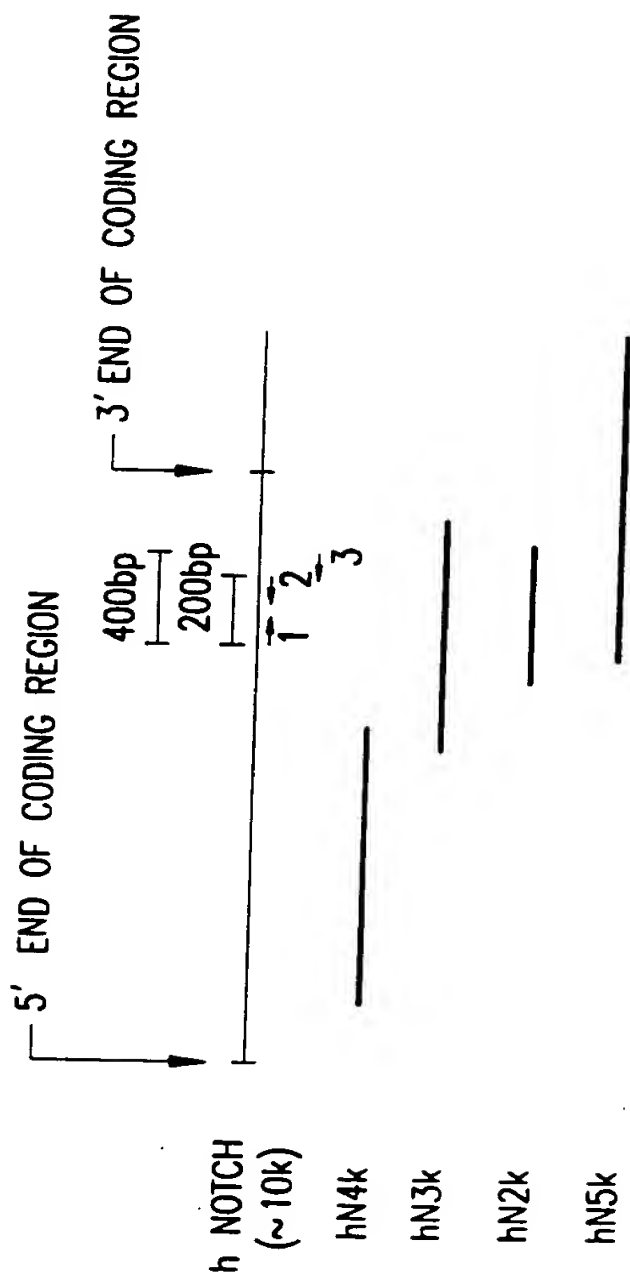
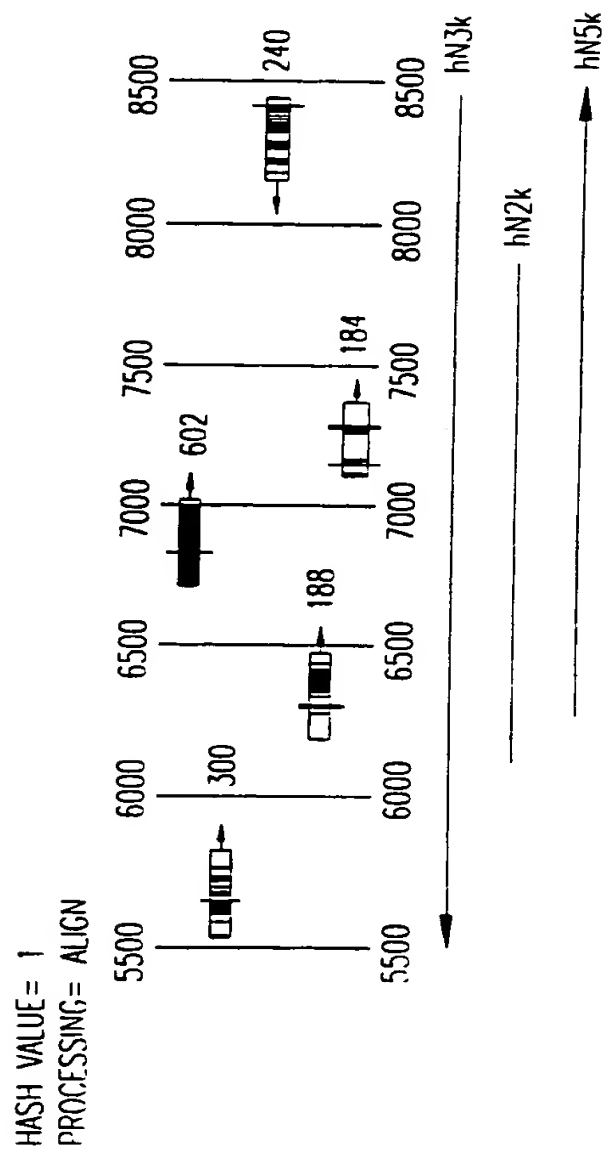
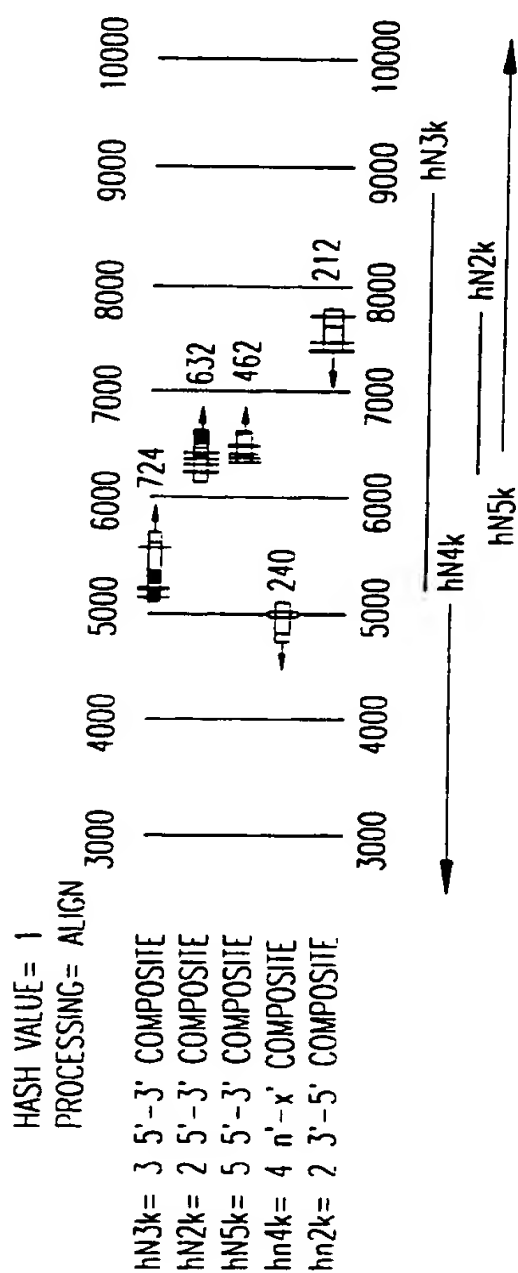


FIG.6

FIG. 7



15/68

1 GAATTCCGCT GGGAGAATGG TCTGAGCTAC CTGCCCCGTCC TGCTGGGGCA TCAATGGCAA
61 GTGGGGAAAG CCACACTGGG CAAACGGGCC AGGCCATTTC TGGAAATGTGG TACATGGTGG
121 GCAGGGGGCC CGCAACAGCT GGAGGGCAGG TGGACTGAGG CTGGGGATCC CCCGCTGGTT
181 GGGCAATACT GCCTTTACCC ATGAGCTGGA AAGTCACAAT GGGGGGCAAG GGCTCCCGAG
241 GGTGGTTATG TGCTTCCTTC AGGTGGC

FIG.8A

1 GAATTCCTTC CATTATACGT GACTTTTCTG AACTGTAGC CACCCTAGTG TCTCTAACTC
61 CCTCTGGAGT TTGTCAGCTT TGGTCTTTTC AAAGAGCAGG CTCTCTTCAA GCTCCTTAAT
121 GCGGGCATGC TCCAGTTTGG TCTGCGTCTC AAGATCACCT TTGGTAATTG ATTCTTCTTC
181 AACCCGGAAC TGAAGGCTGG CTCTCACCCCT CTAGGCAGAG CAGGAATTCC GAGGTGGATG
241 TGTTAGATGT GAATGTCCGT GGCCCAGATG GCTGCACCCC ATTGATGTTG GCTTCTCTCC
301 GAGGAGGCAG CTCAGATTTG AGTGATGAAG ATGAAGATGC AGAGGACTGT TCTGCTAACA
361 TCATCACAGA CTTGGTCTAC CAGGGTGCCA GCCTCCAGAC CAGACAGACC GGAAGGTGGA
421 GATGGCCCTG CACCTTGCA GCGGCTACTC ACGGGCTGAT GCTGCCAAGC GTCTCCTGGA
481 TGCAGGTGCA GATGCCAATG CCCAGGACAA CATGGGCCGC TGCTCACTCC ATGCTGCAGT
541 GGCACGTGAT GCCAAGGTGT ATTCAGATCT GTTA

FIG.8B

1 TCCAGATTCT GATTCCGAAC CGAGTAACTG ATCTAGATGC CAGGATGAAT GATGGTACTA
61 CACCCCTGAT CCTGGCTGCC CGCCTGGCTG TGGAGGGAAT GGTGGCAGAA CTGATCAACT
121 GCCAAGCGGA TGTGAATGCA GTGGATGACC ATGGAAAATC TGCTCTTCAC TGGGCAGCTG
181 CTGTCAATAA TGTGGAGGCA ACTCTTTTGT TGTTGAAAAA TGGGGCCAAC CGAGACATGC
241 AGGACAACAA GGAAGAGACA CCTCTGTTTC TTGCTGCCCC GGAGGAGCTA TAAGC

FIG.8C

16/68

1 GAATTCATT CAGGAGGAAA GGGTGGGGAG AGAAGCAGGC ACCCACTTTC CCGTGGCTGG
61 ACTCGTTCCT AGGTGGCTCC ACCGGCAGCT GTGACCGCCG CAGGTGGGGG CCGAGTGCCA
121 TTCAGAAAAT TCCAGAAAAG CCTACCCCA ACTCGGACGG CAACGTCACA CCGTGGGTA
181 GCAACTGGCA CACAAACAGC CAGCGTGTCT GGGGCACGGG GGGATGGCAC CCCCTGCAGG
241 CAGAGCTG

FIG.9A

1 CTAAAGGGAA CAAAAGCNGG AGCTCCACCG CGGGCGGCNC NGCTCTAGAA CTAGTGGANN
61 NCCCGGGCTG CAGGAATTC GGGGACTGG GCTCGGGCTC AGAGCGGCGC TGTGGAAGAG
121 ATTCTAGACC GGGAGAACAA GCGAATGGCT GACAGCTGGC CTCAAAGTC ACCAGGCTCA
181 AATCGCTCGC CCTGGACATC GAGGGATGCA GAGGATCAGA ACCGGTACCT GGATGGCATG
241 ACTCGGATTT ACAAGCATGA CCAGCCTGCT TACAGGGAGC GTGANNTTTT CACATGCAGT
301 CGACAGACAC GAGCTCTATG CAT

FIG.9B

17/68

```

10  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
    TGC CAG GAG GAC GCG GGC AAC AAG GTC TGC AGC CTG CAG TGC AAC AAC
    C   Q   E   D   A   G   N   K   V   C   S   L   Q   C   N   N>

50  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
    CAC GCG TGC GGC TGG GAC GGC GGT GAC TGC TCC CTC AAC TTC AAT GAC
    H   A   C   G   W   D   G   G   D   C   S   L   N   F   N   D>

100 *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
    CCC TGG AAG AAC TGC ACG CAG TCT CTG CAG TGC TGG AAG TAC TTC AGT
    P   W   K   N   C   T   Q   S   L   Q   C   W   K   Y   F   S>

150 *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
    GAC GGC CAC TGT GAC AGC CAG TGC AAC TCA GCC GGC TGC CTC TTC GAC
    D   G   H   C   D   S   Q   C   N   S   A   G   C   L   F   D>

```

FIG. 10A

18/68

```

200      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      GGC TTT GAC TGC CAG CGT GCG GAA GGC CAG TGC AAC CCC CTG TAC GAC      *      *      *
      G   F   D   C   Q   R   A   E   G   Q   C   N   P   L   Y   D> -
210      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
220      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
230      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
240      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
250      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      CAG TAC TGC AAG GAC CAC TTC AGC GAC GGC CAC TGC GAC CAG GGC TGC      *      *      *
      Q   Y   C   K   D   H   F   S   D   G   H   C   D   Q   G   C>
260      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
270      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
280      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
290      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      AAC AGC GCG GAG TGC GAG TGG GAC GGC CTG GAC TGT GCG GAG CAT GTA      *      *      *
      N   S   A   E   C   E   W   D   G   L   D   C   A   E   H   V>
300      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
310      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
320      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
330      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
340      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      CCC GAG AGG CTG GCG GCC GGC ACG CTG GTG GTG GTG CTG ATG CCG      *      *      *
      P   E   R   L   A   A   G   T   L   V   V   V   V   L   M   P>
350      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
360      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
370      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
380      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *

```

FIG.10B

* CCG GAG CAG CTG CGC AAC AGC TCC TTC CAC TTC CTC CGG GAG CTC AGC
P E Q L R N S F H F L R E L S> 410 * 420 * 430 *

* CCG GTG CTG CAC ACC AAC GTG GTC TTC AAG CGT GAC GCA CAC GCC CAG
R V L H T N V F K R D A H G Q> 440 * 450 * 460 * 470 * 480 *

* CAG ATG ATC TTC CCC TAC TAC GGC CGC GAG GAG GAG CTG CGC AAG CAC
Q M I F P Y Y G R E E E L R K HD> 490 * 500 * 510 * 520 *

* CCC ATC AAG CGT GCC GCC GAG GGC TGG GCC GCA CCT GAC GCC CTG CTG
P I K R A A E G W A A P D A L LD> 530 * 540 * 550 * 560 * 570 *

FIG. 10C

20/68

```

580      *      *      *      *      *      *      *      *
      GGC CAG GTG AAG GCC TCG CTG CTC CCT GGT GGC AGC GAG GGT GGG CGG
      G  Q  V  K  A  S  L  L  P  G  G  S  E  G  G  R>
      610      620

630      *      *      *      *      *      *      *      *
      CGG CGG AGG GAG CTG GAC CCC ATG GAC GTC CGC GGC TCC ATC GTC TAC
      R  R  R  E  L  D  P  M  D  V  R  G  S  I  V  Y>
      640      650      660      670

680      *      *      *      *      *      *      *      *
      CTG GAG ATT GAC AAC CGG CAG TGT GTG CAG GCC TCC TCG CAG TGC TTC
      L  E  I  D  N  R  Q  C  V  Q  A  S  S  Q  C  F>
      690      700      710      720

730      *      *      *      *      *      *      *      *
      CAG AGT GCC ACC GAC GTG GCC GCA TTC CTG GGA GCG CTC GCC TCG CTG
      Q  S  A  T  D  V  A  A  F  L  G  A  L  A  S  L>
      740      750      760

```

FIG. 10D

21/68

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770      *      780      *      790      *      800      *      810      *
      GGC AGC CTC AAC ATC CCC TAC AAG ATC GAG GCC GTG CAG AGT GAG ACC
      G  S  L  N  I  P  Y  K  I  E  A  V  Q  S  E  T>-
      820      830      840      850      860
      GTG GAG CCG CCC CCG CCG CAG CTG CAC TTC ATG TAC GTG GCG GCG
      V  E  P  P  P  A  Q  L  H  F  M  Y  V  A  A>
      870      880      890      900      910
      *      *      *      *      *
      GCC GCC TTT GTG CTT CTG TTC TTC GTG GGC TGC GGG GTG CTG CTG TCC
      A  A  F  V  L  L  F  F  V  G  C  G  V  L  L  S>
      920      930      940      950      960
      *      *      *      *      *
      CGC AAG CGC CGG CAG CAT GGC CAG CTC TGG TTC CCT GAG GGC TTC
      R  K  R  R  Q  H  G  Q  L  W  F  P  E  G  F>

```

FIG.10E

22/68

```

970      *      *      *      *      *      *      *      *      *      *
AAA GTG TCT GAG GCC AGC AAG AAG AAG CGG CGG GAG CCC CTC GGC GAG
K   V   S   E   A   S   K   K   K   R   R   E   P   L   G   E>

1010     *      *      *      *      *      *      *      *      *      *
      1020     1030     1040     1050
GAC TCC GTG GGC CTC AAG CCC CTG AAG AAC GCT TCA GAC GGT GCC CTC
D   S   V   G   L   K   P   L   K   N   A   S   D   G   A   L>

1060     *      *      *      *      *      *      *      *      *      *
      1070     1080     1090     1100
ATG GAC GAC AAC CAG AAT GAG TGG GGG GAC GAG GAC CTG GAG ACC AAG
M   D   D   N   Q   N   E   W   G   D   E   D   L   E   T   K>

1110     *      *      *      *      *      *      *      *      *      *
      1120     1130     1140     1150
AAG TTC CGG TTC GAG GAG CCC GTG GTT CTG CCT GAC CTG GAC GAC CAG
K   F   R   F   E   E   P   V   V   L   P   D   L   D   D   Q>

```

FIG.10F

23/68

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1160      +      1170      +      1180      +      1190      +      1200      +
ACA GAC CAC CGG CAG TGG ACT CAG CAG CAC CTG GAT GCC GCT GAC CTG
T   D   H   R   Q   W   T   Q   Q   H   L   D   A   A   D   L>

1210      1220      1230      1240
*      +      *      +
CGC ATG TCT GCC ATG GCC CCC ACA CCG CCC CAG GGT GAG GTT GAC GCC
R   M   S   A   M   A   P   T   P   P   Q   G   E   V   D   A>

1250      1260      1270      1280      1290
+      +      +      +      +
GAC TGC ATG GAC GTC AAT GTC CGC GGC CCT GAT GGC TTC ACC CCG CTC
D   C   M   D   V   N   V   R   G   P   D   G   F   T   P   L>

1300      1310      1320      1330      1340
*      +      +      +      +
ATG ATC GCC TCC TGC AGC GGC GGC GGC CTG GAG ACG GGC AAC AGC GAG
M   I   A   S   C   S   G   G   G   L   E   T   G   N   S   E>

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FIG.10G

24/68

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1350      *      1360      1370      1380      1390
      *      *      *      *      *
GAA GAG GAG GAC GCG CCG GCC GTC ATC TCC GAC TTC ATC TAC CAG GGC
E   E   E   D   A   P   A   V   I   S   D   F   I   Y   Q   G>

1400      1410      1420      1430      1440
      *      *      *      *      *
GCC AGC CTG CAC AAC CAG ACA GAC CGC ACG GGC GAG ACC GCC TTG CAC
A   S   L   H   N   Q   T   D   R   T   G   E   T   A   L   HD>

1450      1460      1470      1480
      *      *      *      *
CTG GCC GCC CGC TAC TCA CGC TCT GAT GCC GCC AAG CGC CTG CTG GAG
L   A   A   R   Y   S   R   S   D   A   A   A   K   R   L   L   E>

1490      1500      1510      1520      1530
      *      *      *      *      *
GCC AGC GCA GAT GCC AAC ATC CAG GAG AAC ATG GGC CGC ACC CCG CTG
A   S   A   D   A   N   I   Q   D   N   M   G   R   T   P   L>

```

FIG. 10H

25/68

```

1540      *      1550      *      1560      *      1570      *      1580      *
CAT GCG GCT GTG TCT GCC GAC GCA CAA GGT GTC TTC CAG ATC CTG ATC
H   A   A   V   S   A   D   A   Q   G   V   F   Q   I   L   I>

1590      *      1600      *      1610      *      1620      *      1630      *
CGG AAC CGA GCC ACA GAC CTG GAT GCC CGC ATG CAT GAT GGC ACG ACG
R   N   R   A   T   D   L   D   A   R   M   H   D   G   T   T>

1640      *      1650      *      1660      *      1670      *      1680      *
CCA CTG ATC CTG GCT GCC CGC CTG GCC GTG GAG GGC ATG CTG GAG GAC
P   L   I   L   A   A   R   L   A   V   E   G   M   L   E   D>

1690      *      1700      *      1710      *      1720      *
CTC ATC AAC TCA CAC GCC GAC GTC AAC GCC GTA GAT GAC CTG GGC AAG
L   I   N   S   H   A   D   V   N   A   V   D   D   L   G   K>

1730      *      1740      *      1750      *      1760      *      1770      *
TCC GCC CTG CAC TGG GCC GCC GCG GTG AAC AAT GTG GAT GCC GCA GTT
S   A   L   H   W   A   A   A   V   N   N   V   D   A   A   V>

```

FIG. 10I

26/68

1780	1790	1800	1810	1820
★	★	★	★	★
GTG CTC CTG AAG AAC GGG GCT AAC AAA GAT ATG CAG AAC AAC AGG GAG				
V L L K N G A N K D M Q N R E>				
1830	1840	1850	1860	1870
★	★	★	★	★
GAG ACA CCC CTG TTT CTG GCC GCC CGG GAG GGC AGC TAC GAG ACC GCC				
E T P L F L A A R E G S Y E T A>				
1880	1890	1900	1910	1920
★	★	★	★	★
AAG GTG CTG CTG GAC CAC TTT GCC AAC CGG GAC ATC ACG GAT CAT ATG				
K V L L D H F A N R D I T D H M>				
1930	1940	1950	1960	
★	★	★	★	
GAC CGC CTG CCG CGC GAC ATC GCA CAG GAG CGC ATG CAT CAC GAC ATC				
D R L P R D I A Q E R M H D I>				

FIG.10J

27/68

1970	1980	1990	2000	2010
★	★	★	★	★
GTG AGG CTG CTG GAC GAG TAC AAC CTG GTG CGC AGC CCG CAG CTG CAC				
V R L L D E Y N L V R S P Q L HD				
2020	2030	2040	2050	2060
★	★	★	★	★
GGA GCC CCG CTG GGG GGC ACG CCC ACC CTG TCG CCC CCG CTC TGC TCG				
G A P L G G T P T L S P P L C S>				
2070	2080	2090	2100	2110
★	★	★	★	★
CCC AAC GGC TAC CTG GGC AGC CTC AAG CCC GGC GTG CAG GGC AAG AAG				
P N G Y L G S L K P G V Q G K K>				
2120	2130	2140	2150	2160
★	★	★	★	★
GTC CGC AAG CCC AGC AGC AAA GGC CTG GCC TGT GGA AGC GAG GCC				
V R K P S S K G G L A C G S K E A>				

FIG. 10K

28/68

2170	2180	2190	2200
★ AAG GAC CTC AAG GCA CGG AGG AAG AAG TCC CAG GAT GGC AAG GGC TGC	★	★	★
K D L K A R R K R K S Q D G K G C>			
2210	2220	2230	2240
★ CTG CTG GAC AGC TCC GGC ATG CTC TCG CCC GTG GAC TCC CTG GAG TCA	★	★	★
L L D S S G M L S P V D S L E S>			
2260	2270	2280	2290
★ CCC CAT GGC TAC CTG TCA GAC GTG GCC TCG CCG CCA CTG CTG CCC TCC	★	★	★
P H G Y L S D V A S P P L L P S>			
2310	2320	2330	2340
★ CCG TTC CAG CAG TCT CCG TCC GTG CCC CTC AAC CAC CTG CCT GGG ATG	★	★	★
P F Q Q S P S V P L N H L P G MD			

FIG.10L

29/68

2360	2370	2380	2390	2400
* CCC GAC ACC CAC CTG GGC ATC GGC CAC CTG AAC GTG GCC GCC AAG CCC P D T H L G I G H L N V A A K P>	2410	2420	2430	2440
* GAG ATG GCG GCG CTG GGT GGC GGC GGC CTG GCC TTT GAG ACT GGC E M A A L G G G G G R L A F E T G>	2450	2460	2470	2480
* CCA CCT CGT CTC TCC CAC CTG CCT CTG GGC TCT GGC ACC AGC ACC GTC P P R L S H L P V A S G T S T V>	2490	2500	2510	2520
* CTG GGC TCC AGC AGC GGA GGC GCC CTG AAT TTC ACT GTG GGC GGC TCC L G S S S G G A L N F T V G G S>	2530	2540	2550	2560

FIG.10M

30/68

```

2550  * 2560 2570 2580 2590
      *  *  *  *  *
ACC AGT TTG AAT GGT CAA TGC GAG TGG CTG TCC CGG CTG CAG AGC GGC
T   S   L   N   G   Q   C   E   W   L   S   R   L   Q   S   G>

2600 2610 2620 2630 2640
      *  *  *  *  *
ATG GTG CCG AAC CAA TAC AAC CCT CTG CCG GGG AGT GTG GCA CCA GGC
M   V   P   N   Q   Y   N   P   L   R   G   S   V   A   P   G>

2650 2660 2670 2680
      *  *  *  *
CCC CTG AGC ACA CAG GCC CCC TCC CTG CAG CAT GGC ATG GTA GGC CCG
P   L   S   T   Q   A   P   S   L   Q   H   G   M   V   G   P>

2690 2700 2710 2720 2730
      *  *  *  *  *
CTG CAC AGT AGC CTT GCT GCC AGC GCC CTG TCC CAG ATG ATG AGC TAC
L   H   S   S   L   A   A   S   A   L   S   Q   M   M   S   Y>

```

FIG. 10N

31/68

2740 * 2750 2760 2770 2780
 CAG GGC CTG CCC AGC ACC CGG CTG GCC ACC CAG CCT CAC CTG GTG CAG
 Q G L P S T R L A T Q P H L V Q>
 * * * * *
 2790 2800 2810 2820 2830
 ACC CAG CAG GTG CAG CCA CAA AAC TTA CAG ATG CAG CAG AAC CTG
 T Q Q V Q P Q N L Q M Q Q N L>
 * * * * *
 2840 2850 2860 2870 2880
 CAG CCA GCA AAC ATC CAG CAG CAA AGC CTG CAG CCG CCA CCA CCA
 Q P A N I Q Q Q S L Q P P P>
 * * * * *
 2890 2900 2910 2920
 CCA CCA CAG CCG CAC CTT GGC GTG AGC TCA GCA GCC AGC GGC CAC CTG
 P P Q P H L G V S S A A S G H L>
 * * * * *
 2930 2940 2950 2960 2970
 GGC CCG AGC TTC CTG AGT GGA GAG CCG AGC CAG GCA GAC GTG CAG CCA
 G R S F L S G E P S Q A D V Q P>
 * * * * *

FIG.100

32/68

2980	2990	3000	3010	3020
CTG GGC CCC AGC AGC CTG GCG GTG CAC ACT ATT CTG CCC CAG GAG AGC				
L G P S S L A V H T I L P Q E S>				
3030	3040	3050	3060	3070
CTG GGC CCC AGC TCG TCG CCA TCC TCG CTG GTC CCA CCC GTG ACC				
P A L P T S L P S S L V P P V T>				
3080	3090	3100	3110	3120
GCA GCC CAG TTC CTG ACG CCC CCC TCG CAG CAC AGC TAC TCC TCG CCT				
A A Q F L T P P S Q H S Y S P>				

FIG.10P

33/68

3130	3140	3150	3160
* GTG GAC AAC ACC CCC AGC CAC CAG CTA CAG GTG CCT GTT CCT GTA ATG	* * * *	* * * *	* * * *
V D N T P S H Q L Q V P V P V M>			
3170	3180	3190	3200
* * * *	* * * *	* * * *	* * * *
GTA ATG ATC CGA TCT TCG GAT CCT TCT AAA GGC TCA ATT TTG ATC			
V M I R S S D P S K G S I L I>			
3220	3230		
* * *	* *		
GAA GCT CCC GAC TCA TGG			
E A P D S W>			

FIG.10Q

34/68

G GAG GTG GAT GTG TTA GAT GTG AAT GTC CGT GGC CCA GAT GGC TGC Glu Val Asp Val Leu Asp Val Asn Val Arg Gly Pro Asp Gly Cys 1 5 10 15	46
ACC CCA TTG ATG TTG GCT TCT CTC CGA GGA GGC AGC TCA GAT TTG AGT Thr Pro Leu Met Leu Ala Ser Leu Arg Gly Gly Ser Ser Asp Leu Ser 20 25 50	94
GAT GAA GAT GAA GAT GCA GAG GAC TCT TCT GCT AAC ATC ATC ACA GAC Asp Glu Asp Glu Asp Ala Glu Asp Ser Ser Ala Asn Ile Ile Thr Asp 35 40 45	142
TTG GTC TAC CAG GGT GCC AGC CTC CAG GCC CAG ACA GAC CGG ACT GGT Leu Val Tyr Gln Gly Ala Ser Leu Gln Ala Gln Thr Asp Arg Thr Gly 50 55 60	190
GAG ATG GCC CTG CAC CTT GCA GCC CGC TAC TCA CGG GCT GAT GCT GCC Glu Met Ala Leu His Leu Ala Ala Arg Tyr Ser Arg Ala Asp Ala Ala 65 70 75	238
AAG CGT CTC CTG GAT GCA GGT GCA GAT GCC AAT GCC CAG GAC AAC ATG Lys Arg Leu Leu Asp Ala Gly Ala Asp Ala Asn Ala Gln Asp Asn Met 80 85 90 95	286
GGC CGC TGT CCA CTC CAT GCT GCA GTG GCA GCT GAT GCC CAA GGT GTC Gly Arg Cys Pro Leu His Ala Ala Val Ala Ala Asp Ala Gln Gly Val 100 105 110	334
TTC CAG ATT CTG ATT CGC AAC CGA GTA ACT GAT CTA GAT GCC AGG ATG Phe Gln Ile Leu Ile Arg Asn Arg Val Thr Asp Leu Asp Ala Arg Met 115 120 125	382
AAT GAT GGT ACT ACA CCC CTG ATC CTG GCT GCC CGC CTG GCT GTG GAG Asn Asp Gly Thr Thr Pro Leu Ile Leu Ala Ala Arg Leu Ala Val Glu 130 135 140	430

FIG.11A

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35/68

GGA ATG GTG GCA GAA CTG ATC AAC TGC CAA GCG GAT GTG AAT GCA GTG	478
Gly Met Val Ala Glu Leu Ile Asn Cys Gln Ala Asp Val Asn Ala Val	
145 150 155	
GAT GAC CAT GGA AAA TCT GCT CTT CAC TGG GCA GCT GCT GTC AAT AAT	526
Asp Asp His Gly Lys Ser Ala Leu His Trp Ala Ala Ala Val Asn Asn	
160 165 170 175	
GTG GAG GCA ACT CTT TTG TTG TTG AAA AAT GGG GCC AAC CGA GAC ATG	574
Val Glu Ala Thr Leu Leu Leu Leu Lys Asn Gly Ala Asn Arg Asp Met	
180 185 190	
CAG GAC AAC AAG GAA GAG ACA CCT CTG TTT CTT GCT GCC CGG GAG GGG	622
Gln Asp Asn Lys Glu Glu Thr Pro Leu Phe Leu Ala Ala Arg Glu Gly	
195 200 205	
AGC TAT GAA GCA GCC AAG ATC CTG TTA GAC CAT TTT GCC AAT CGA GAC	670
Ser Tyr Glu Ala Ala Lys Ile Leu Leu Asp His Phe Ala Asn Arg Asp	
210 215 220	
ATC ACA GAC CAT ATG GAT CGT CTT CCC CGG GAT GTG GCT CGG GAT CGC	718
Ile Thr Asp His Met Asp Arg Leu Pro Arg Asp Val Ala Arg Asp Arg	
225 230 235	
ATG CAC CAT GAC ATT GTG CGC CTT CTG GAT GAA TAC AAT GTG ACC CCA	766
Met His His Asp Ile Val Arg Leu Leu Asp Glu Tyr Asn Val Thr Pro	
240 245 250 255	
AGC CCT CCA GGC ACC GTG TTG ACT TCT GCT CTC TCA CCT GTC ATC TGT	814
Ser Pro Pro Gly Thr Val Leu Thr Ser Ala Leu Ser Pro Val Ile Cys	
260 265 270	
GGG CCC AAC AGA TCT TTC CTC AGC CTG AAG CAC ACC CCA ATG GGC AAG	862
Gly Pro Asn Arg Ser Phe Leu Ser Leu Lys His Thr Pro Met Gly Lys	
275 280 285	

FIG.11B

36/68

AAG TCT AGA CGG CCC AGT GCC AAG AGT ACC ATG CCT ACT AGC CTC CCT	910
Lys Ser Arg Arg Pro Ser Ala Lys Ser Thr Met Pro Thr Ser Leu Pro	
290 295 300	
AAC CTT GCC AAG GAG GCA AAG GAT GCC AAG GGT AGT AGG AGG AAG AAG	958
Asn Leu Ala Lys Glu Ala Lys Asp Ala Lys Gly Ser Arg Arg Lys Lys	
305 310 315	
TCT CTG AGT GAG AAG GTC CAA CTG TCT GAG AGT TCA GTA ACT TTA TCC	1006
Ser Leu Ser Glu Lys Val Gln Leu Ser Glu Ser Ser Val Thr Leu Ser	
320 325 330 335	
CCT GTT GAT TCC CTA GAA TCT CCT CAC ACG TAT GTT TCC GAC ACC ACA	1054
Pro Val Asp Ser Leu Glu Ser Pro His Thr Tyr Val Ser Asp Thr Thr	
340 345 350	
TCC TCT CCA ATG ATT ACA TCC CCT GGG ATC TTA CAG GCC TCA CCC AAC	1102
Ser Ser Pro Met Ile Thr Ser Pro Gly Ile Leu Gln Ala Ser Pro Asn	
355 360 365	
CCT ATG TTG GCC ACT GCC GCC CCT CCT GCC CCA GTC CAT GCC CAG CAT	1150
Pro Met Leu Ala Thr Ala Ala Pro Pro Ala Pro Val His Ala Gln His	
370 375 380	
GCA CTA TCT TTT TCT AAC CTT CAT GAA ATG CAG CCT TTG GCA CAT GGG	1198
Ala Leu Ser Phe Ser Asn Leu His Glu Met Gln Pro Leu Ala His Gly	
385 390 395	
GCC AGC ACT GTG CTT CCC TCA GTG AGC CAG TTG CTA TCC CAC CAC CAC	1246
Ala Ser Thr Val Leu Pro Ser Val Ser Gln Leu Leu Ser His His His	
400 405 410 415	
ATT GTG TCT CCA GGC AGT GGC AGT GCT GGA AGC TTG AGT AGG CTC CAT	1294
Ile Val Ser Pro Gly Ser Gly Ser Ala Gly Ser Leu Ser Arg Leu His	
420 425 430	
CCA GTC CCA GTC CCA GCA GAT TGG ATG AAC CGC ATG GAG GTG AAT GAG	1342
Pro Val Pro Val Pro Ala Asp Trp Met Asn Arg Met Glu Val Asn Glu	
435 440 445	

FIG.11C

37/68

ACC CAG TAC AAT GAG ATG TTT GGT ATG GTC CTG GCT CCA GCT GAG GGC 1390
 Thr Gln Tyr Asn Glu Met Phe Gly Met Val Leu Ala Pro Ala Glu Gly
 450 455 460

ACC CAT CCT GGC ATA GCT CCC CAG AGC AGG CCA CCT GAA GGG AAG CAC 1438
 Thr His Pro Gly Ile Ala Pro Gln Ser Arg Pro Pro Glu Gly Lys His
 465 470 475

ATA ACC ACC CCT CGG GAG CCC TTG CCC CCC ATT GTG ACT TTC CAG CTC 1486
 Ile Thr Thr Pro Arg Glu Pro Leu Pro Pro Ile Val Thr Phe Gln Leu
 480 485 490 495

ATC CCT AAA GGC AGT ATT GCC CAA CCA GCG GGG GCT CCC CAG CCT CAG 1534
 Ile Pro Lys Gly Ser Ile Ala Gln Pro Ala Gly Ala Pro Gln Pro Gln
 500 505 510

TCC ACC TGC CCT CCA GCT GTT GCG GGC CCC CTG CCC ACC ATG TAC CAG 1582
 Ser Thr Cys Pro Pro Ala Val Ala Gly Pro Leu Pro Thr Met Tyr Gln
 515 520 525

ATT CCA GAA ATG GCC CGT TTG CCC AGT GTG GCT TTC CCC ACT GCC ATG 1630
 Ile Pro Glu Met Ala Arg Leu Pro Ser Val Ala Phe Pro Thr Ala Met
 530 535 540

ATG CCC CAG CAG GAC GGG CAG GTA GCT CAG ACC ATT CTC CCA GCC TAT 1678
 Met Pro Gln Gln Asp Gly Gln Val Ala Gln Thr Ile Leu Pro Ala Tyr
 545 550 555

CAT CCT TTC CCA GCC TCT GTG GGC AAG TAC CCC ACA CCC CCT TCA CAG 1726
 His Pro Phe Pro Ala Ser Val Gly Lys Tyr Pro Thr Pro Pro Ser Gln
 560 565 570 575

CAC AGT TAT GCT TCC TCA AAT GCT GCT GAG CGA ACA CCC AGT CAC AGT 1774
 His Ser Tyr Ala Ser Ser Asn Ala Ala Glu Arg Thr Pro Ser His Ser
 580 585 590

GGT CAC CTC CAG GGT GAG CAT CCC TAC CTG ACA CCA TCC CCA GAG TCT 1822
 Gly His Leu Gln Gly Glu His Pro Tyr Leu Thr Pro Ser Pro Glu Ser
 595 600 605

FIG.11D

38/68		
CCT GAC CAG TGG TCA AGT TCA TCA CCC CAC TCT GCT TCT GAC TGG TCA Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser Ala Ser Asp Trp Ser 610 615 620		1870
GAT GTG ACC ACC AGC CCT ACC CCT GGG GGT GCT GGA GGA GGT CAG CGG Asp Val Thr Thr Ser Pro Thr Pro Gly Gly Ala Gly Gly Gly Gln Arg 625 630 635		1918
GGA CCT GGG ACA CAC ATG TCT GAG CCA CCA CAC AAC AAC ATG CAG GTT Gly Pro Gly Thr His Met Ser Glu Pro Pro His Asn Asn Met Gln Val 640 645 650 655		1966
TAT GCG TGAGAGAGTC CACCTCCAGT GTAGAGACAT AACTGACTTT TGTAATGCT Tyr Ala		2022
GCTGAGGAAC AAATGAAGGT CATCCGGGAG AGAAATGAAG AAATCTCTGG AGCCAGCTTC		2082
TAGAGGTAGG AAAGAGAAGA TGTTCCTTATT CAGATAATGC AAGAGAAGCA ATTCGTCAGT		2142
TTCACTGGGT ATCTGCAAGG CTTATTGATT ATTCTAATCT AATAAGACAA GTTTGTGGAA		2202
ATGCAAGATG AATACAAGCC TTGGGTCCAT GTTACTCTC TTCTATTTGG AGAATAAGAT		2262
GGATGCTTAT TGAAGCCCAG ACATTCTTGC AGCTTGGACT GCATTTTAAG CCCTGCAGGC		2322
TTCTGCCATA TCCATGAGAA GATTCTACAC TAGCGTCCTG TTGGGAATTA TGCCCTGGAA		2382
TTCTGCCTGA ATTGACCTAC GCATCTCCTC CTCCTTGGAC ATTCTTTTGT CTTCAATTTGG		2442
TGCTTTTGGT TTTGCACCTC TCCGTGATTG TAGCCCTACC AGCATGTTAT AGGGCAAGAC		2502
CTTTGTGCTT TTGATCATTC TGGCCCATGA AAGCAACTTT GGTCTCCTTT CCCCTCCTGT		2562
CTTCCCGGTA TCCCTTGGAG TCTCACAAGG TTTACTTTGG TATGGTTCTC AGCACAACC		2622
TTTCAAGTAT GTTGTTTCTT TGGAAAATGG ACATACTGTA TTGTGTTCTC CTGCATATAT		2682
CATTCCTGGA GAGAGAAGGG GAGAAGAATA CTTTCTTCA ACAAATTTTG GGGGCAGGAG		2742
ATCCCTTCAA GAGGCTGCAC CTTAATTTTT CTGTCTGTG TGCAGGTCTT CATATAAACT		2802

FIG.11E

SUBSTITUTE SHEET (RULE 26)

39/68

TTACCAGGAA GAAGGGTGTG AGTTTGTGT TTTTCTGTGT ATGGGCCTGG TCAGTGTA	2862
TTTATCCT TGATAGTCTA GTTACTATGA CCCTCCCCAC TTTTAAAA CCAGAAAAG	2922
TTTGAATG TTGGAATGAC CAAGAGACAA GTTAACTCGT GCAAGAGCCA GTTACCCACC	2982
CACAGGTCCC CCTACTTCCT GCCAAGCATT CCATTGACTG CCTGTATGGA ACACATTGT	3042
CCCAGATCTG AGCATTCTAG GCCTGTTTCA CTCCTCACC CAGCATATGA AACTAGTCTT	3102
AACTGTTGAG CCTTTCCTTT CATATCCACA GAAGACACTG TCTCAAATGT TGTACCCTTG	3162
CCATTAGGA CTGAACCTTC CTTAGCCCAA GGGACCCAGT GACAGTTGTC TTCCGTTTGT	3222
CAGATGATCA GTCTCTACTG ATTATCTTGC TGCTTAAAGG CCTGCTCACC AATCTTCTT	3282
TCACACCGTG TGGTCCGTGT TACTGGTATA CCCAGTATGT TCTCACTGAA GACATGGACT	3342
TTATATGTTT AAGTGCAGGA ATTGGAAAGT TGGACTTGTT TTCTATGATC CAAAACAGCC	3402
CTATAAGAAG GTTGGAAAAG GAGGAACTAT ATAGCAGCCT TTGCTATTTT CTGCTACCAT	3462
TTCTTTTCCT CTGAAGCGGC CATGACATTC CCTTTGGCAA CTAACGTAGA AACTCAACAG	3522

FIG.11F

40/68

AACATTTTCC TTTCCTAGAG TCACCTTTTA GATGATAATG GACAACTATA GACTTGCTCA	3582
TTGTTTCTAGAC TGATTGCCCC TCACCTGAAT CCACTCTCTG TATTCATGCT CTTGGCAATT	3642
TCTTTGACTT TCTTTTAAGG GCAGAAGCAT TTTAGTTAAT TGTAAGATAAA GAATAGTTTT	3702
CTTCCTCTTC TCCTTGGGCC AGTTAATAAT TGGTCCATGG CTACACTGCA ACTTCCGTCC	3762
AGTGCTGTGA TGCCCATGAC ACCTGCAAAA TAAGTTCTGC CTGGGCATTT TGTAAGATATT	3822
AACAGGTGAA TTCCCGACTC TTTTGGTTTG AATGACAGTT CTCATTCTTT CTATGGCTGC	3882
AAGTATGCAT CAGTGCTTCC CACTTACCTG ATTTGTCTGT CCGTGGCCCC ATATGGAAAC	3942
CCTGCGTGTG TGTTGGCATA ATAGTTTACA AATGGTTTTT TCAGTCCTAT CCAAATTTAT	4002
TGAACCAACA AAAATAATTA CTTCCTGCCCT GAGATAAGCA GATTAAGTTT GTTCATTCTC	4062
TGCTTTATTC TCTCCATGTG GCAACATTCT GTCAGCCTCT TTCATAGTGT GCAACATTT	4122
TATCATTCTA AATGGTGACT CTCTGCCCTT GGACCCATTT ATTATTCACA GATGGGGAGA	4182
ACCTATCTGC ATGGACCCTC ACCATCCTCT GTGCAGCACA CACAGTGCAG GGAGCCAGTG	4242
GCGATGGCGA TGACTTTCTT CCCCTG	4268

FIG. 11G

SUBSTITUTE SHEET (RULE 26)

41/68

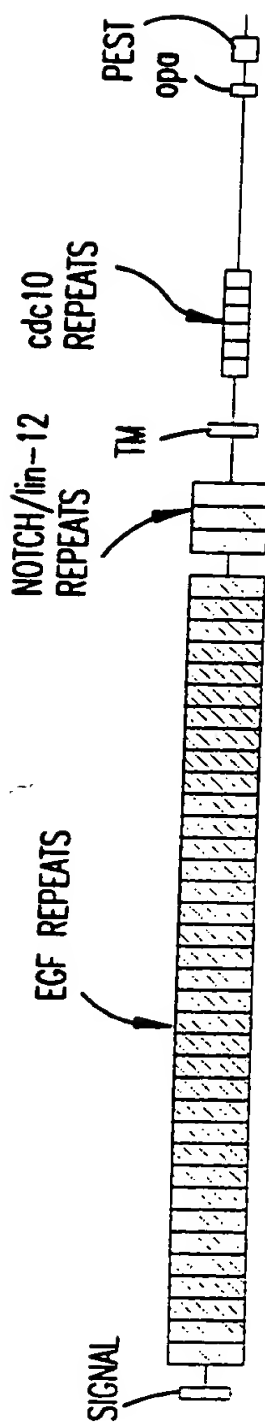


FIG. 12A

42/68

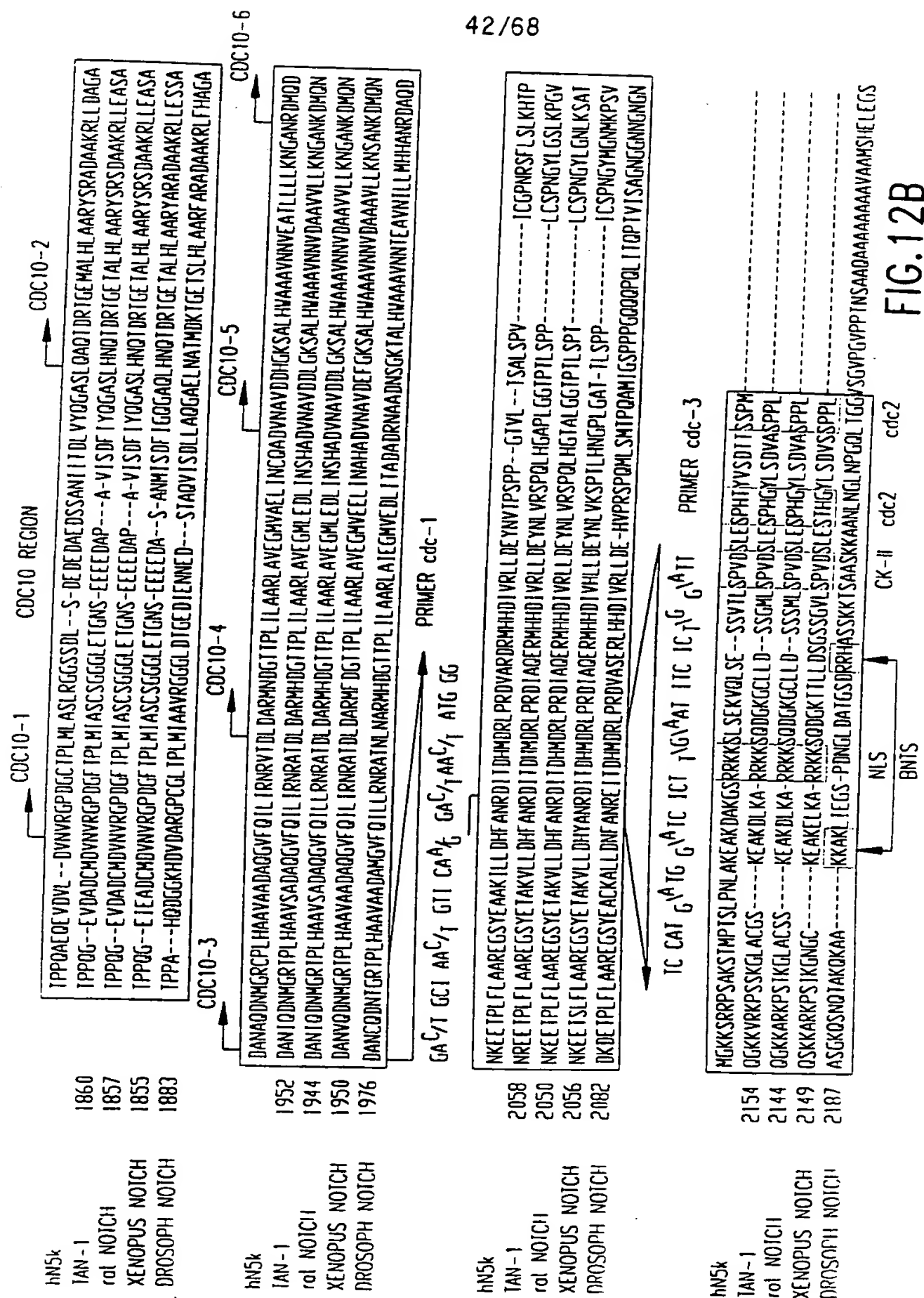


FIG. 12B

hN5k			
IAN-1	2218	-----	-----
rat NOTCH	2209	-----	-----
XENDPUS NOTCH	2214	-----	-----
DRISOPH NOTCH	2285	-----	-----

hNSk	SNLHEHQ-----	PLAHGASTVLPSVSQLLSHHHIVSPGS--	GSAGSL SRLHPVPVPADV--	MNRHEVNE TOYNEFMGMV APAEG-THPGI	CK-II
TAN-1	2250	A-KPEMAALGGGRLAFTGPPRLSHLPVASTITVLGSSGGALNFTVGGSTSLNGQCEVL SRLQSGMVPNDYNPLRGSVAPGPLSTQAPSLQHG-MVGPLHSSL			
rot NOTCH	2242	A-KPEMAALAGGSRLAFTPPPPRLSHLPVASSASTVLSTNGTGAMNFTVGAPASLNGQCEVL PRLQNGMVPQYNPLRPQVTPGTLSTQAAGLQHGMM-SPJHSSL			
XENDPUS NOTCH	2247	T-KQCHAA--GSNRMAFDAMVPRLTIL-NASSPNTIMS---NGSMHFTVGGAPIMNSQCDWLARLQNGMVGNDQYDPIRNGTQQGN-AQDADALQHG LMTS-LHNGL			
DRUSDPH NOTCH	2390	GGLCCHGGGLSGAGNGNSHEOGLSPYPYS-NQSPPHSVQSSALSPHAYLGPSPSPAKSRPSLPTSPTHIQAMRHTAQKQFGGSNLNSLLGGANGGVVGGGGGGGGV			

[illegible]

hN5k		SVAFPTAHMPQDDGVAQTILPAYHPFPASVCKYHIIPPSQHSYASSNAERTPSHSGHLQGEHPYLIPSPESPQDWSSSPHSA-SDWSDVTTISPT
TAN-I	2448	SSLAVHTILPQ-ESPALPTSLPSSLVPPVTAQFLIPPSQHSY-SS-PVENTPSHQLQVP-EHPFLIPSPESPQDWSSSPHSNVDWSEGVSSPPT
rot NOTCH	2423	SSLPVHTILPQ-ESQALPTSLPSSWPPPHITQFLIPPSQHSY-SSSPVDNTPSHQLQVP-EHPFLIPSPESPQDWSSSPHSNVDWSEGVSSPPT
XENDUPUS NOTCH	2416	SSNNIHSVMPQ-DTQIFAAASLPNLTQSHITAQFLIPPSQHSY-SS-PMDNTPSHQLQVP-DHPFLIPSPESPQDWSSSPHSNVDWSEGVSSPPT
DRISOPH NOTCH	2599	S---IQSSMSG-SSPSTINMLSPSPINDDAQYQYIIPSSQHS-----GGHTPQHLVQTL-D-SYPIPSPESPGHWSSSSPSRN-SDWSEGVQSPAA

PEST-CONTAINING REGION

FIG. 12C

44/68

		Potential signal cleavage site									
hum N	MP	—	—	—	ALRPAL	LWALLALWLC	CA	—	APA	HA	—
TAN-1	MP	—	—	—	PL	LAPLLCLALL	PA	—	LAA	RG	—
Xen N	MD	—	—	—	RIGLAVLLCS	LP	—	VLT	QG	—	—
Dros N	MOSQSRRRRS	RAPNTWICFW	INKMHAVASL	PASLPLLLLT	LAFANLPNIV	RGTDTALVAA					
hum N	MLGKATCRCA	SGFTGEDCQY	STSHPCFVSR	PCLNGGTCHM	LSRDT-YECT	CQVGTGKEC					
Tan-1	GVADYACSCA	LGFSGPLCLT	PLDNAC-LTN	PCRNGGTCOL	LT-LTEYKCR	CPPGWSGKSC					
Xen N	NAIDFICHCP	VGFTDKVCLT	PVDNAC-VNN	PCRNGGTCEL	LNSVTEYKCR	CPPGWTGDSC					
Dros N	GRPGISCKCP	LGFDLSLCEI	AVPNAC-DHV	TCLNGGTCOL	KT-LEEYTC	CANGYTGERC					
hum N	NLPGSYQCQC	PQGTGQYCD	SLYVPCAPSP	CVNGGTCROT	GDFTFECNCL	PGFEGSTCER					
TAN-1	NEVGSYRCVC	RATHTGPNCE	RPYVPCSPSP	CONGGTCRPT	GDVTHEACCL	PGFTGQNCCE					
Xen N	NEFGSYRCTC	QNRFTGRNCD	EPYVPCNPSP	CLNGGTCROT	DDTSYDCTCL	PGFSGQNCCE					
Dros N	NTHGSYQCMC	PTGYTGKDCD	TKYNPCSPSP	CONAGICRSN	G-LSYECKCP	KGFEGKNCEQ					

EGF-like Repeats

QCRDGYEPCV	NEGMCVITYHN	GTGYCKPEG	FLGEYCQHRD	PCE-KNRCQN	GGTC—VAQA	83
RCSQGETCL	NKGKCEA-AN	GTEACVCGGA	FVGPCCODPN	PCL-STPCKN	AGTCHVDDRR	80
RCTOTAEMCL	NGGRCEMTPG	GTGVCLCGNL	YFGERCQFPN	PCTIKNQCMN	FGTCEPVLOQ	90
SCTSVG—CQ	NGGTCVTQLN	GKTYCACDSH	YVG DYCEHRN	PCN-SMRCQN	GGTCQVTFRN	117
QWTDACLSP	CANGSTCTTV	—ANQFSCKC	LIGFTGQKCE	TDVNEC-DIP	GHCOHGGTCL	199
QOADPCASNP	CANGGQCLPF	—EASYICHC	PPSFHGPTCR	QDVNECGQKP	RLCRHGGTCH	196
QOADPCASNP	CANGGKCLPF	—EIQYICKC	PPGFHGATCK	QDINEC-S-Q	NPCKNGGQCI	195
ETKNLCASSP	CRNGATCTAL	AGSSSFTCS	PPGFTGDTCS	YDIEEC-Q-S	NPCKYGGICV	233
NIDDCPNHRC	QNGGVCVDGV	NTYNCRCPPO	WTGQFCTEDV	DECLLPNA-	CONGGTCANR	318
NIDDCPGNNC	KNGGACVDGV	NTYNCPPE	WTGQYCTEDV	DECQLMPNA-	CONGGTCHNT	315
NIDDCPSNNC	RNGGTCVDGV	NTYNCCPPD	WTGQYCTEDV	DECQLMPNA-	CONGGTCHNT	314
NYDDCLGHL	CNGGTCIDGI	SDYTCRCPN	FTGRFCODDV	DECAQRDHPV	CONGATCTNT	352

FIG.13A

45/68

hum N	NGGYGCVVN	GWSGDDCSEN	IDDCAFASCT	PGSTCIDRVA	SFSCMCPEGK	AGLLCHLDDA
TAN-1	HGGYNCVCVN	GTGEDCSEN	IDDCASAACF	HGATCHDRVA	SFYCECPHGR	TGLLCHLNDA
Xen N	YGGYNCVCVN	GTGEDCSEN	IDDCANAACH	SGATCHDRVA	SFYCECPHGR	TGLLCHLDNA
Dros N	HGSYSCICVN	GWAGLDCSNN	TDDCKQAACF	YGATCIDGVG	SFYCQCTKGK	TGLLCHLDDA

hum N	AFHCECLKGY	AGPRCEMDIN	ECHSDPCQND	ATCLDKITGGF	TCLCMPGFKG	VHCELEINEC
TAN-1	SFECQCLQGY	TGPRCEIDVN	ECVSNPCQND	ATCLDQIGEF	OCMCMPGYEG	VHCEVNTDEC
Xen N	SFQCNCPOGY	AGPRCEIDVN	ECLSNPCQND	STCLDQIGEF	QCICMPGYEG	LYCETNIDEC
Dros N	SYRCNCSQGF	TGPRCETNIN	ECESHPCQNE	GSCLDDPGTF	RCVCMPTGTF	TQCEIDIDEC

hum N	ATGFTGVLCE	ENIDNCDPDP	CHHGQCQDGI	DSYTCICNPG	YMGATCSDQI	DECYSSPCLN
TAN-1	TEGYTGTHCE	VDIDECDDP	CHYGSCCKGV	ATFTCLCRPG	YTGHHCEINI	NECSSQPCRL
Xen N	TEGFTGRHCE	QDINECIPDP	CHYGTCKDGI	ATFTCLCRPG	YTGRLCDNDI	NECLSKPCLN
Dros N	PPGYTGTSC	ININDCDSNP	CHRGKCIDDV	NSFKCLCDPG	YTGICOKQI	NECESNPCOF

CISNPCHKGA	LCDTNPLNGQ	YICTCPQGYK	GADCTEDVDE	CAMANSNPCE	HAGKCVNTDG	438
CISNPCNEGS	NCDTNPVNGK	AICTCPSGYT	GPACSDQVDE	CSLG-ANPCE	HAGKCINTLG	434
CISNPCNEGS	NCDTNPVNGK	AICTCPPGYT	GPACNNDVDE	CSLG-ANPCE	HGGRCNTNLG	433
CTSNPCHADA	ICDTSPINGS	YACSCATGYK	GVDCSEDIDE	CDQG-SPCE	HNGICVNTPG	470

QSNPCVNNGQ	CVDKVNRFQC	LCPPGFTGPV	CQIDIDDCSS	TPCLNGAKCI	DHPNGYECQC	558
ASSPCLHNGR	CLDKINEFQC	ECPTGFTGHL	COYDVDECAS	TPCKNGAKCL	DGPNTYTCVC	554
ASNPCLHNGK	CIDKINEFRC	DCPTGFSGNL	CQHDFDECTS	TPCKNGAKCL	DGPNSYTCQC	553
QSNPCLNDGT	CHDKINGFKC	SCALGFTGAR	CQINIDDCQS	QPCRNRGICH	DSIAGYSCEC	590

DGRCIDLUNG	YQCNCQPGTS	GVNCEINFDD	CASNPCIHG-	ICMDGINRYS	CVCSPGFTGQ	677
RGTCQDPDPA	YLCFCLKGTT	GPNCENLDD	CASSPCDSG-	TCLDKIDGYE	CACEPGYTGS	673
GGQCTDRENG	YICTCPKGTT	GVNCETKIDD	CASNLCQNG-	KCIDKIDGYE	CTCEPGYTGK	672
DGHCQDRVGS	YYCQCQAGTS	GKNCEVNVNE	CHSNPCNNGA	TCIDGINSYK	CQCVPFTGQ	710

FIG.13B

46/68

hum N	RCNIDIDECA	SNPCRKGATC	INGVNGFRCI	CPEGPHHPSC	YSQVNECLSN	PCI-HGNCCTG
TAN-1	MCNSNIDECA	GNPCHNGGTC	EDGINGFTCR	CPEGYHDPTC	LSEVNECNSN	PCV-HGACRD
Xen N	LCNININECD	SNPCRNGGTC	KDQINGFTCV	CPDGYDHMC	LSEVNECNSN	PCI-HGACHD
Dros N	HCEKNVDECI	SSPCANNGVC	IDQVNGYKCE	CPRGFYDAHC	LSDVDECASN	PCVNEGRCD
hum N	DECASNPCLN	QGTCFDDISG	YTCHCVLPYT	GKNCQTVLAP	CSPNPCENAA	VCKESPNFES
TAN-1	NECASNPCLN	KGTCIDDVAG	YKCNCLLPYT	GATCEVVLAP	CAPSPCRNGG	ECROSEDYES
Xen N	NECSSNPCLN	HGTCIDDVAG	YKCNCLLPYT	GAICEAVLAP	CAGSPCKNGG	RCKESEDFT
Dros N	DDCVTNPCGN	GGTCIDKVNG	YKCVCKVPFT	GRDCESKMDP	CASNRCKNEA	KCTPSSNFLD
hum N	CLANPCQNGG	SCMDGVNTFS	CLCLPGFTGD	KCQTDNMECL	SEPCKNGGTC	SDYVNSYTCK
TAN-1	CRPNPCHNGG	SCTDGINAF	CDCLPGFRGT	FCEEDINECA	SDPCRNGANC	TDCVDSYTCT
Xen N	CQPNPCHNGG	SCSDGINMFF	CNCPAGFRGP	KCEEDINECA	SNPCKNGANC	TDCVNSYTCT
Dros N	CASFPCQNGG	TCLDGIGDYS	CLCVDGFDGK	HCETDINECL	SQPCQNGATC	SQYVNSYTCT

GLSGYKCLCD	AGWVGINCEV	DKNECLSNPC	QNGGTCNVLV	NGYRCTCKKG	FKGYNCQVNI	796
SLNGYKDCD	PGWSGTNCDI	NNNECESNPC	VNGGTCKDMT	SGIVCTCREG	FSGPNCQTNI	792
GVNGYKDCD	AGWSGSNCDI	NNNECESNPC	MNGGTCKDMT	GAYICTCKAG	FSGPNCQTNI	791
GINEFICHCP	PGYTGRCEL	DIDECSSNPC	QHGGTCYDKL	NAFSCQCMPC	YTGQKCETNI	830

YTCLCA-PGW	QGQRTIDID	EC-ISKPCM	HGLCHNTQGS	YMCECPPGFS	GMDCEEDIDD	914
FSCVCPTAG	KGQTCVDIN	EC-VLSPCRH	GASCONTHGG	YRCHCOAGYS	GRNCETDIDD	911
FSCECP-PGW	QGQTCIDMN	EC-VNRPCRN	GATCONTHGS	YKCNCKPGYT	GRNCMDIDD	909
FSCTCK-LGY	TGRYCEDID	ECSLSSPCRN	GASCLNVPGS	YRCLCTKGYE	GRDCAINTDD	949

CQAGFDGVHC	ENNINECTES	SCFNGGTCVD	GINSFSLCP	VGFTGSFCLH	EINECSSHPC	1034
CPAGFSGIHC	ENNTPDCTES	SCFNGGTCVD	GINSFTCLCP	PGFTGSYCOH	VVNECDSRPC	1031
CQPGFSGIHC	ESNTPDCTES	SCFNGGTCID	GINTFTCQCP	PGFTGSYCOH	DINECDSPKC	1029
CPLGFSGINC	QTNDEDCTES	SCLNGGSCID	GINGYNCSC	AGYSGANCQY	KLKNCDSNPC	1069

FIG.13C

SUBSTITUTE SHEET (RULE 26)

47/68

hum N	LNEGTCVDGL	GTYRCSCPLG	YTGKNCQTLV	NLCRSPPCKN	KGTCVQKKA	EQCLCPSGWA
TAN-1	LLGGTCODGR	GLHRCTCPQG	YTGPNCONLV	HWCDSSPCKN	GGKCWQHTQ	YRCECPSGWT
Xen N	LNGGTCQDSY	GTWKCTCPQG	YTGLNCONLV	RWCDSSPCKN	GGKCWQTNF	YRCECKSGWT
Dros N	LNGATCHEQN	NEYTCHCPSG	FTGKOCSEYV	DWCGQSPCEN	GATCSQMKHQ	FCKCSAGWT

hum N	SNPCQHGATC	SDFIGGYRCE	CVPGYQGVNC	EYEVDECONQ	PCQNGGTCID	LVNHFKCSCP
TAN-1	PSPCQNGATC	TDYLGYSCK	CVAGYHGVNC	SEEIDECLSH	PCQNGGTCLD	LPNTYKCS
Xen N	PNPCQNGATC	TDYLGYSCE	CVAGYHGVNC	SEEINECLSH	PCQNGGTCID	LINTYKCS
Dros N	SQPCQNGGTC	RDLIGAYECQ	CROGFGQNC	ELNIDDCAPN	PCQNGGTCHD	RVMNFCSCP

hum N	CLSNPCSSEG	SLDCIQLTND	YLCVCRSAFT	GRHCETFDV	CPQMPCLNGG	TCAVASNMPD
TAN-1	CLSNPCDARG	TQNCVQRVND	FHCECRAGHT	GRRCESVING	CKGKPKNGG	TCAVASNTAR
Xen N	CLSNPCDSRG	TQNCIQLVND	YRCECROGFT	GRRCESVDG	CKGMPCRNNG	TCAVASNTER
Dros N	CLSNPCSNA	TLDCVQLVNN	YHCNCRPGHM	GRHCEHKVDF	CAQSPCQNGG	NCNI—RQS

GAYCDVPNV	CDIAASRRGV	LVEHLCOHSG	VCINAGNTHY	CQCPLGYTGS	YCEEQLDECA	1154
GLYCDVPSV	CEVAAORQGV	DVARLCOHGG	LCVDAGNTHH	CRQAGYTGS	YCEDLVDECS	1151
GYYCDVPSV	CEVAAKQGGV	DIVHLCRNSG	MCVDTGNTHF	CRQAGYTGS	YCEEQVDECS	1149
GKLCDVQTI	CDAAADRKGL	SLRQLC—NNG	TCKDYGNSHV	CYCSQGYAGS	YQKEIDECQ	1188

PGTRGLLEE	NIDDCAR—	—GPHCLN	GGQCMDRIGG	YSCRCLPGFA	GERCEGDINE	1267
RGTQGVHCE	IVDDCNPPVD	PVSRSPKCFN	NGTCVDOVGG	YSCTCPPGFV	GERCEGDVNE	1271
RGTQGVHCE	IVDDCTPFYD	SFTLEPKCFN	NGKCIDRVGG	YNCICPPGFV	GERCEGDVNE	1269
PMTGIIICE	NKDDCKP—	—GACHN	NGSCIDRVGG	FECVCQPGFV	GARCEGDINE	1300

GFICRCPPGF	SGARCQS—	SGQVKCRKG	EQCVHTAS—	GPRCFCPSP—	—RDCE—	1376
GFICKCPAGF	EGATCENDAR	TCGSLRCLNG	GTCISGPR—	SPTCLCLGPF	TCPECQFPAS	1389
GFICKCPPGF	DGATCEYDSR	TCSNLRCONG	GTCISVLT—	SSKVCSEGY	TGATCQYPVI	1387
GHHICNNGF	YGKNCLESGQ	DCDSNPCRVG	—NCVVADEGF	GYRCECPRGT	LGEHCEIDTL	1415

FIG.13D

48/68

hum N	-GC-ASSPCQ HGGSCHPQRQ PPYYSCQCAP PFGSRCEL- -YTAPP- -S- -TPP
TAN-1	SPCLGGNPCY NOGTCEPTSE SPFYRCLCPA KFNGLLCHIL DYSFGG- -GAGRDIPPP
Xen N	SPC-ASHPCY NGGTCQFFAE EPFFQCFCPK NFNGLFCHIL DYEFG- -GLGKNITPP
Dros N	DEC-SPNPCA QGAACEDLLG D-YECLCPS KWKGRCDIY DANYPGWNGG SGSCNDRYAA

hum N	NN-QCDELGN TVECLFDNFE CQNSKTCK- -YDKYCADHF KDNHCNQCEN SEECGWGGLD
TAN-1	SDGHCDSCN SAGCLFDGFD CORAEGQCNP LYDQYCKDHF SDGHCDQGCN SAECEWDGLD
Xen N	NDGKCDSCN NTGCLYDGF DCKVEQCNP LYDQYCKDHF QDGHCDQGCN NAECEWDGLD
Dros N	KNGKCNEECN NAACHYDGH CERKLKSCDS LFDAYCQKHY GDGFCDYGCN NAECSWDGLD

hum N	YYGEKSAAMK KQ-R- -MTRRSL PGEQ- -E QEVAGSKVFL
TAN-1	YYGREEELRK HPIKRAAEGW AAPDALLQOV KASLLPGGSE GRRRRRELDP MDVRGSIVYL
Xen N	YYGNEEELKK HHIKRSTDYD SDAPSAI- -FSTMKESIL LGRHRRELDE MEVRGSIVYL
Dros N	WKDNVRVPEI EDTDFARKNK ILYTQQVHQ- -TGIIQIYL

LNR (Notch/Lin-12 Repeats)

-A- TCL	SOYCADKARD GVCDEACNSH ACQWDGGDCS LTMENPWANC SSPLPCWDYI	1476
LIEE- ACE	LPEQEDAGN KVCSLQCNH ACQWDGGDCS LNFNDPWKNC TQSLQWKYF	1501
DNDD- ICE	NEQCEADN KVCNANCNNH ACQWDGGDCS LNFNDPWKNC TQSLQWKYF	1498
DLEQORAMCD	KRGCTEKQGN GICSDCNTY ACNFDGNDCS LGI-NPWANC TAN-EXWNKF	1531

CAADQOPEN-L	AEGTLVIVVL MPPEQLLODA R-SFLRALGT LLHTNLRIKR DSQELMVYP	1591
CAEHVPER-L	AAGTL-VVVV LMPPEQLRNS SFHFLRELSR VLHTNVVFKR DAHQOOMIFP	1619
C-ANMPEN-L	AEGTLVLVVL MPPERLKNNS V-NFLRELSR VLHTNVVFKK DSKGEYKIYP	1615
CENKTQSPVL	AEGAMSVVML MNVEAFREIQ A-QFLRNMSH MLRTTVRLKK DALGHDIIIN	1650

EIDNRQCVQD	SDHCFKNTDA AAALLASHAI QG-TLSYP LVSVVSESLT PERT-Q-LLY	1680
EIDNRQCVQA	SSQCFOSATD VAAFLGALAS LGSL-NIPYK IEAVQSETVE PPPPAQ-LHF	1737
EIDNRQCYKS	SSQCFNSATD VAAFLGALAS LGSLDTLSYK IEAVKSENME TPKPST-LYP	1730
EIDNRKCTEC	FTHAVEAAEF LAATAAKHQL RNDFO-IHSV RGIKNPGDED NGEPPANVKY	1745

FIG.13E

49/68

hum N	LLAVAVVIL	FIILLGVIMA	KRKRK—HGS	LWLPEGFTLR	RDASNHKRRE	PVGQDAVGLK
TAN-1	MYVAAAFVL	FFVGGCVLL	SRKRRRQHGQ	LWFPEGFKV—	SEASKKKRRE	ELGEDSVGLK
Xen N	MLSMLVIPL	IFVFMVIV	NKKRRREHDS	FGSPTALFOK	NPA—KRNGE	PW—EDSVGLK
Dros N	VITGIILVII	ALAFFGMVL—	STQRKRAHGV	TWFPEGFRAP	AAVMSRRRRD	PHGQEMRNLN

CDC-10/Ankyrin Repeats

hum N	PIDRRPWTOQ	HLEAADIRRT	PSLALTPPQA	EQEVDVLDVN	VRGPDGCTPL	MLASLRGGSS
TAN-1	QTDHROWTOQ	HLDAADL—RM	SAMAPTTPQG	EVDADCMDVN	VRGPDGFTPL	MIASCSGGGL
Xen N	KTDPROWTRQ	HLDAADL—RI	SSMAPTTPQG	EIEADCMDVN	VRGPDGFTPL	MIASCSGGGL
Dros N	EADQRVWSQA	HLDVVDV—R—	AIM—TPP—A	HQDGGKHVDV	ARGPCGLTPL	MIAAVRGGGL

hum N	ANAQDNMGRC	PLHAAVAADA	QGVFQILIRN	RVTDL DARMN	DGTTPLILAA	RLAVEGMVAE
TAN-1	ANIQDNMGRT	PLHAASADA	QGVFQILIRN	RATDL DARMH	DGTTPLILAA	RLAVEGMLED
Xen N	ANVQDNMGRT	PLHAAVAADA	QGVFQILIRN	RATDL DARMF	DGTTPLILAA	RLAVEGMVEE
Dros N	ANCQDNTGRT	PLHAAVAADA	MGVFQILLRN	RATNLNARMH	DGTTPLILAA	RLAIEGMVED

NLSVQVSEAN	LIGTGTSEHW	VDDE—	—	—	G	POPKKVKAED	EALLSE—EDD	1782
PLK—NASDGA	LMDDNONE—W	GDED—	—	—	—	LETKKFRFEE	PWVLPD—LDD	1837
PIK—NMTDGS	FMDNONE—W	GDEET—	—	—	—	LENKRFRFEE	QVILPELVDD	1831
KQVAMQSQGV	GQGAH—W	SDESMDPLP	KRORS	SDPVSG	VGLGNNGGYA	SDHTMVSEYE		1861

DLSDDEDADAE	DSSANIITDL	VYQGASLQAO	TDRIGEMALH	LAARYSRADA	AKRLLDAGAD	1902
ETGNSEEE—E	DAPA—VISDF	IYQGASLHNO	TDRIGETALH	LAARYSRSDA	AKRLLEASAD	1954
ETGNSEEE—E	DASANMISDF	IGQGAQLHNO	TDRIGETALH	LAARYARADA	AKRLLESSAD	1949
DTGEDIENNE	DSTAQVISDL	LAQGAELNAT	MDKTGETSLH	LAARFARADA	AKRLLDAGAD	1976

LINCOADVNA	VDDHGKSALH	WAAAVNNVEA	TLLLLKNGAN	ROMQDNKEET	PLFLAAREGS	2022
LINSHADVNA	VDDLGKSALH	WAAAVNNVDA	AVVLLKNGAN	KDMONNREET	PLFLAAREGS	2074
LINAHADVNA	VDEFGKSALH	WAAAVNNVDA	AAVLLKNSAN	KDMONNKEET	SLFLAAREGS	2069
LITADADINA	ADNSGKTALH	WAAAVNNTEA	VNILLMHAN	RDAQDDKDET	PLFLAAREGS	2096

FIG.13F

50/68

hum N	Y E A A K I L L D H F A N R D I T D H M D R L P R D V A R D R M H H D I V R L L D E Y N V T P S P P — G T V L — T S
TAN-1	Y E T A K V L L D H F A N R D I T D H M D R L P R D I A Q E R M H H D I V R L L D E Y N L V R S P Q L H G A P L G G T P
Xen N	Y E T A K V L L D H Y A N R D I T D H M D R L P R D I A Q E R M H H D I V H L L D E Y N L V K S P T L H N G P L G A T —
Dros N	Y E A C K A L L D N F A N R E I T D H M D R L P R D V A S E R L H H D I V R L L D E — H V P R S P Q M L S M T P Q A M I

	NLS		CK II	cdc2	cdc2
hum N	G S R R K K S L S E K V Q L S E — S S	V T L S P V D S L E	S P H T Y V S D T T	S S P M	— — — — —
TAN-1	A — R R K K S Q D G K G C L L D — S S	G M L S P V D S L E	S P H G Y L S D V A	S P P L	— — — — —
Xen N	A — R R K K S Q D G K T T L L D S G S S	G V L S P V D S L E	S T H G Y L S D V S	S P P L	— — — — —
Dros N	G S — P D N G L D A T G S L R R K A S S	K K T S A A S K K A	A N L N G L N P G Q	L T G G V S G V P G	V P P T N S A A Q A
	B N T S				

hum N	— — — — —	— — — — —	— — — — —	I T S P G I L Q A S	P N P M L — A T A	A P P A P V H A Q H
TAN-1	— — — — —	— — — — —	— — — — —	L P S P F — Q Q S	P S V P L N H L P G	M P D T H L G I G H
Xen N	— — — — —	— — — — —	— — — — —	M T S P F — Q Q S	P S M P L N H L T S	M P E S Q L G M N H
Dros N	Y E D C I K N A Q S	M Q S L Q G N G L D	M I K L D N Y A Y S	M G S P F — Q Q E	L L N G Q G L G M N	G N G Q R N G V G P
	CK II			cdc2		

ALSPV — — — — —	ICGP	N R S F L S L K H T	P M G K K S R R P S	A K S T M P T S L P	N L A K E A K D A K	2127
TLSP — — — — —	LCSP	N G Y L G S L K P G	V O G K K V R K P S	S K G L A C G S — —	K E A K D L K	2178
TLSP — — — — —	ICSP	N G Y M G N M K P S	V O S K K A R K P S	I K G N G C — — — —	K E A K E L K	2170
G S P P P G Q Q Q P	Q L I T Q P T V I S	A G N G G N N G N G	N A S G K Q S N Q T	A K Q K A A — — — —	K K A K L I E	2208

— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	2169
— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	2219
— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	2213
AAAAAAVAA	M S H E L E G S P V	G V G M G G N L P S	P Y D T S S M Y S N	A M A A P L A N G N	P N T G A K Q P P S	2327

AL S F S N L H E M Q — — — — —	— — — — —	— — — — —	— — — — —	— P L A H G A S T V	L P S V S Q L L S H	H H I V S P G S —	2235
L N V A A — K P E M	A A L G G G G R L A	F E T G P P R L S H	L P V A S G T S T V	L G S S S G G A L N	F T V G G S T S L N		2306
I N M A T — K Q E M	A A — G S N R M A	F D A M V P R L T H	L — N A S S P N T I	M S — N G S M H	F T V G G A P T M N		2294
G V L P G G L C G M	G G L S G A G N G N	S H E Q G L S P P Y	S N Q S P P H S V Q	S S L A L S P H A Y	L G S P S P A K S R		2445

FIG.13G

51/68

hum N	GSAGLSRLH PVPVPADW— MNRMEVNETQ YNEMFGMVL PAEG—THPGI APOSRPPEGK
TAN-1	GQCEWLSRLQ SGMVPNQYNP LRGSVAPGPL STQAPSLQHG —MVGPLHSSL AASALSOMMS
Xen N	SQCDWLARLQ NGMVQNYDP IRNGIQOQN— AQAQALQHG LMTS—LHNGL PATTLSOMMT
Dros N	PSLPTSPTHI QAMRHATQOK QFGGSLNSL LGGANGGGVV GGGGGGGGV GGGPQNSPVS
hum N	APQPOSTCPP AVAGPLPTMY QIP——EM ARL—PSVAFP TAMMPQDQ VAQTILPAYH
TAN-1	PPQPHLGVS AASGHLGRSF LSGEPSQADV QPLGPSSLAV HTILPQ—ESP ALPTSLPSSL
Xen N	MQQQHHN—SS TTSTHINSF CSSDISQDQL QQM—SSNNI HSVMPQ—DTQ IFAASLPSNL
Dros N	QQQLGGLEFG SAGLDLNG—F CGSPDSFHSG QMNPPS—I QSSMSG—SSP STNMLSPSSQ
hum N	SDWSDVTTSP TPGGAGGQR GPGTHMSEPPHNN MQVYA
TAN-1	SDWSEGVSSP PT——SMQ SQIARPEAFK
Xen N	SDWSEGISSP PT——SMQ PORTHIPEAFK
Dros N	SDWSEGVQSP AANNLYISGG HQANKGSEAIYI

———	——HITPRE PLPP—IV—TF QLIPKGSIAQ PAG———	2320
———	——YQGLPSTRL ATQPHLVQTQ QVQPONLQMQ QONLQPANIQ QOOSLQPPPP	2414
———	——YOAMPNTRL ANQPHLMQAA QMQQQQN—— ————LQLHQS	2384
LGIIISPTGSD	MGIMLAPPQS SKNSAIMQTI SPQQQQQQQQ QQQQQHQQQQ QQQQQQQQQQ	2565

PEST -containing Region

PFPASVGKYP	TPPSQHSYAS SNAARTPSH SGHLOGEHPY LTPSPESPDQ WSSSSPHSA—	2433
VPPVTAAQFL	TPPSQHSY—S S—PVENTPSH QLQVP—EGPF LTPSPESPDQ WSSSSPHSNV	2530
TQSMITAAQFL	TPPSQHSY—S S—PMONTPSH QLQVP—DHPF LTPSPESPDQ WSSSSPHSNM	2497
HNQQAIFYQL	TPSSQHS—— —GGHTPOH LVQTL—D—SY PTPSPESPGH WSSSSPRSN—	2671

2471
2556
2523
2703

FIG.13H

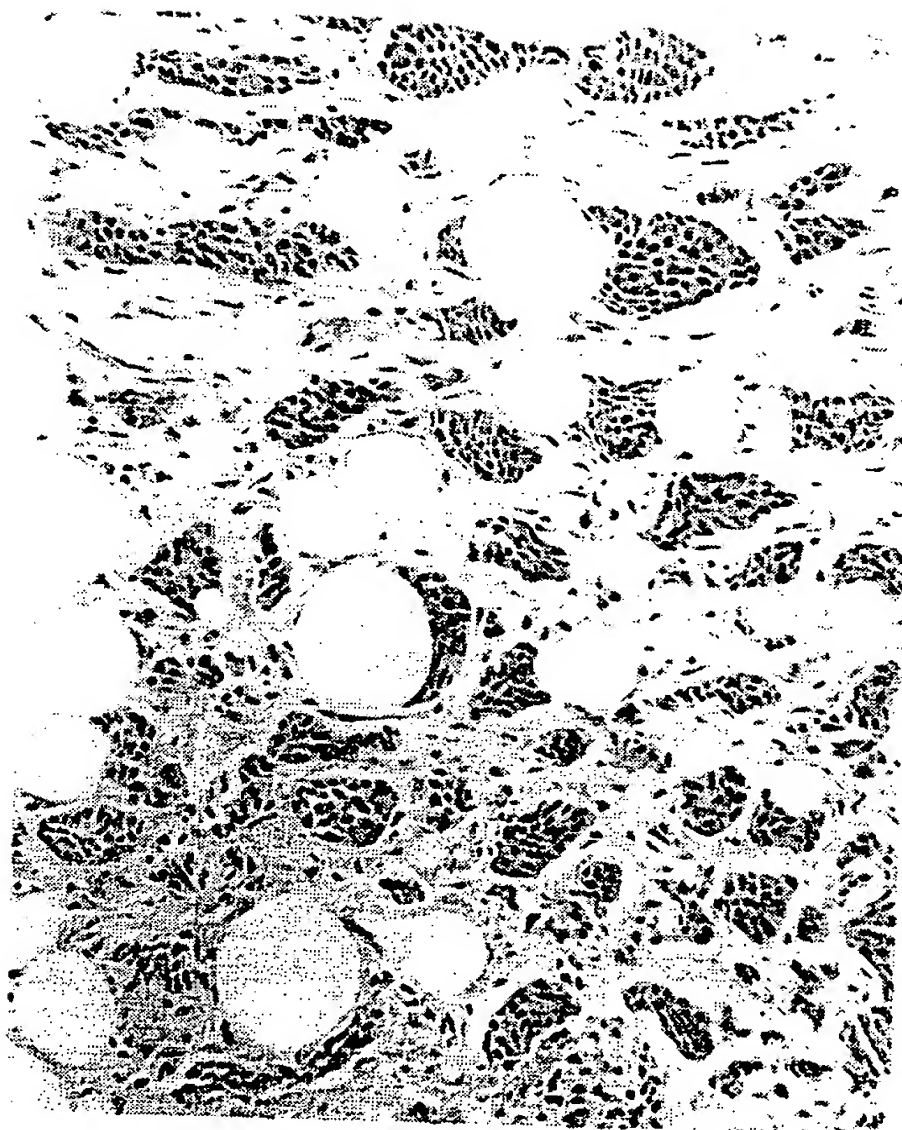


FIG.14

SUBSTITUTE SHEET (RULE 26)

53/68

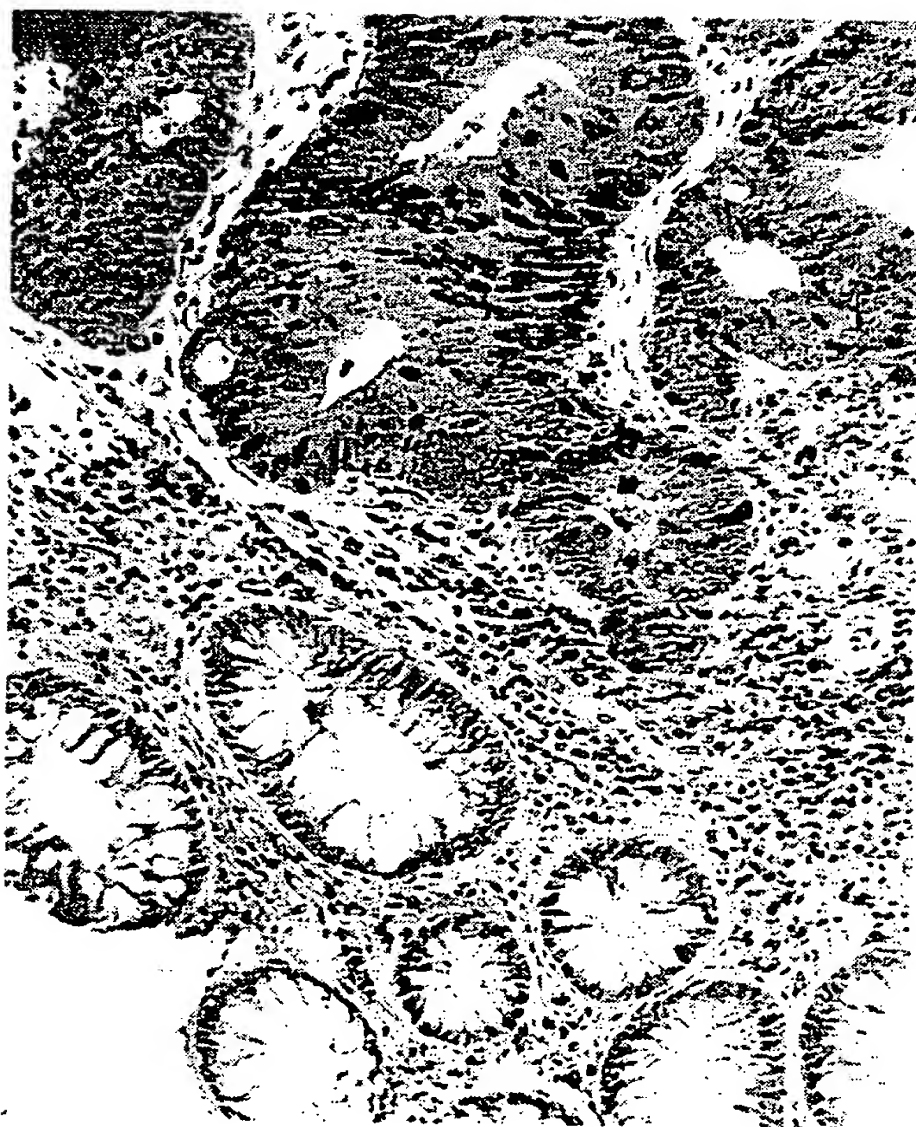


FIG. 15A

54/68

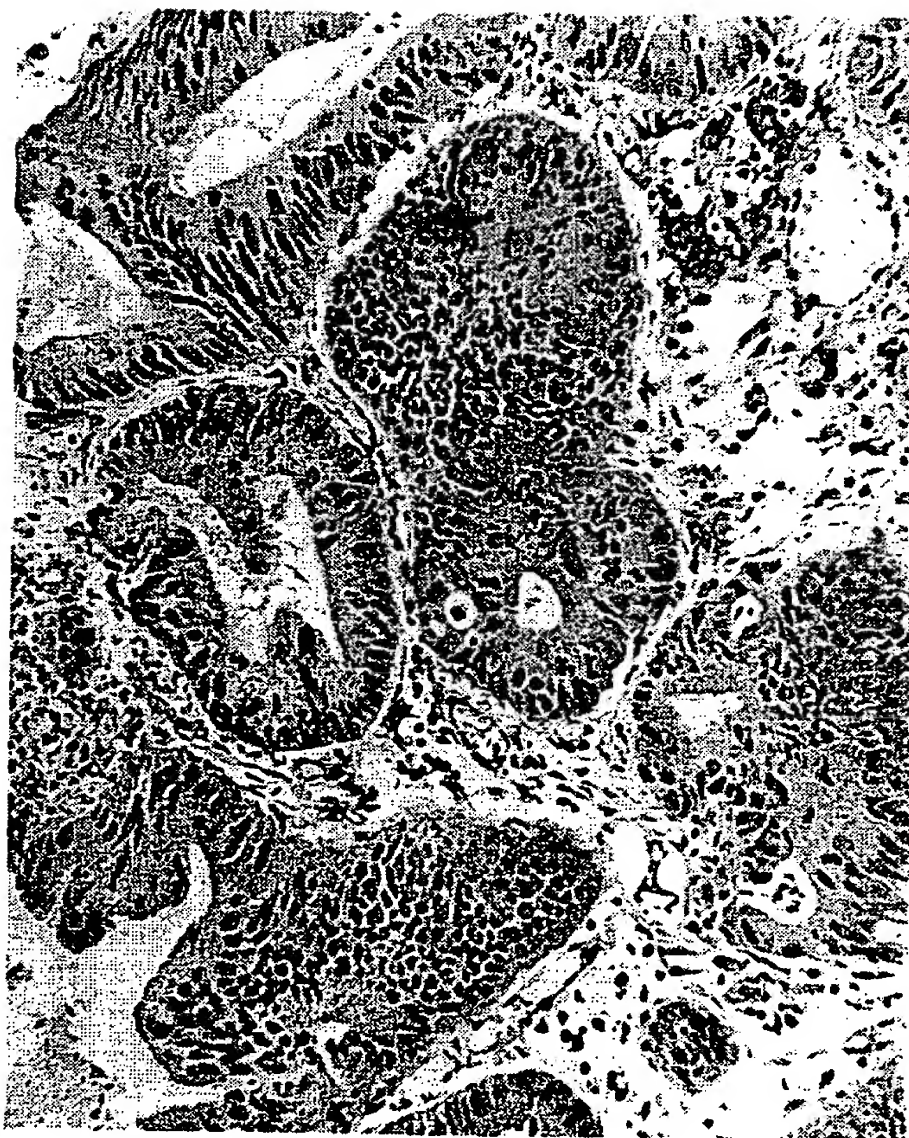


FIG. 15B

SUBSTITUTE SHEET (RULE 26)

55/68

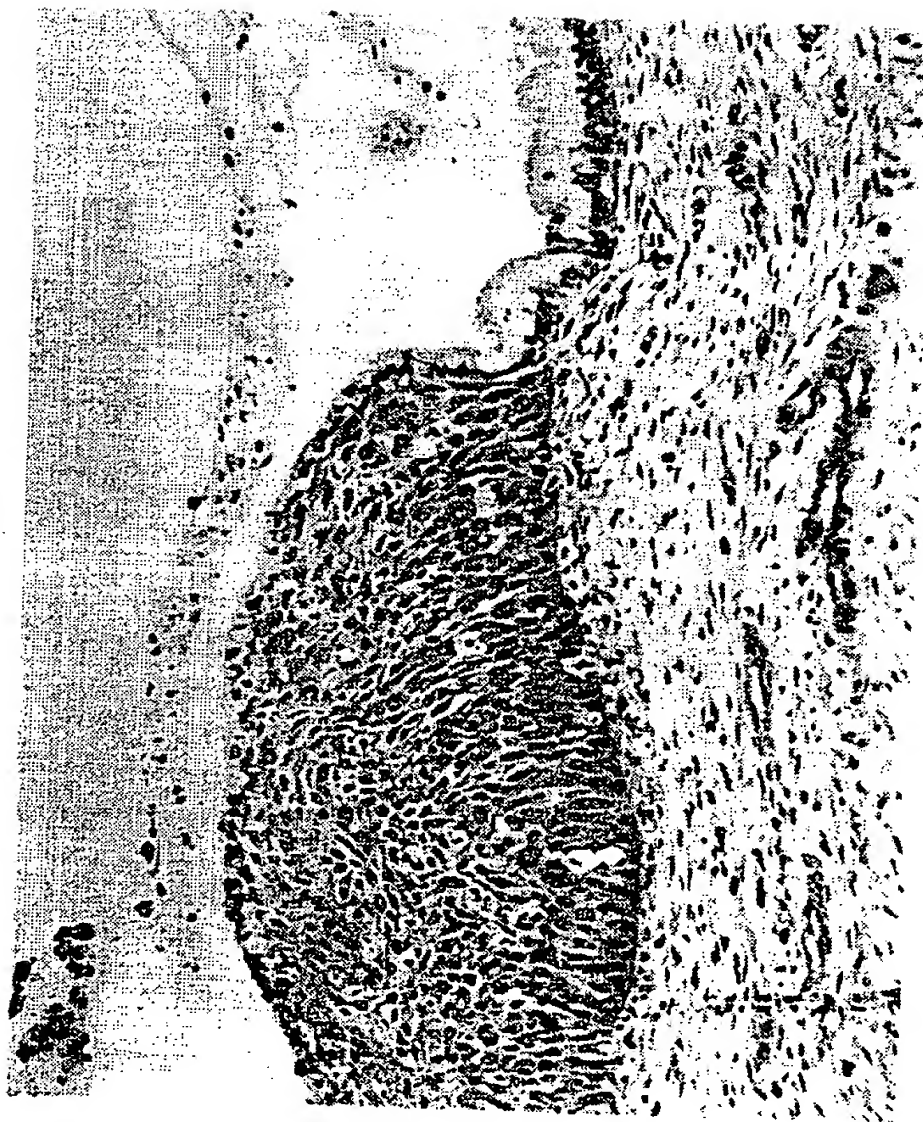


FIG. 16A

56/68



FIG. 16B

SUBSTITUTE SHEET (RULE 26)

57/68

10 20 30 40 50 60 70 80 90
GGAATCCGC CCGCCCTGC CCGGCTCTG CTGTGGGGC TGCTGGCCT CTGGCTGTC TCGCGGGCC CCGGCATGC ATTGCAGTG
P A L R P A L L W A L L A L W L C C A A P A H A L Q C>

100 110 120 130 140 150 160 170 180
CGAGATGGCT ATGAACCTG TGTAATGAA GGAATGTGT TTACCTACCA CAATGGCACA GGATACTGCA AATGTCCAGA AGGCTTCTG
R D G Y E P C V N E G M C V T Y H N G T G Y C K C P E G F L>

190 200 210 220 230 240 250 260 270
GGGAATATT GTCAACATG AGACCCCTG GAGAAGAACC GCTGCCAGAA TGGTGGGACT TGTGTGGCC AGGCCATGCT GGGGAAAGCC
G E Y C Q H R D P C E K N R C Q N G G T C V A Q A M L G K A>

280 290 300 310 320 330 340 350 360
ACGTCCGAT GTGCCTCAGG GTTACAGGA GAGGACTGCC AGTACTCAAC ATCTCATCCA TGCTTTGTGT CTGACCCCTG CCTGAATGGC
T C R C A S G F T G E D C Q Y S T S H P C F V S R P C L N G>

370 380 390 400 410 420 430 440 450
GGCAGATGCC ATATGCTCAG CCGGATACC TATGAGTGCA CCTGTCAAGT CCGGTTTACA GGTAAGGAGT GCCAATGGAC GGATGCCTGC
G T C H M L S R D T Y E C T C Q V G F T G K E C Q W T D A C>

460 470 480 490 500 510 520 530 540
CTGTCTCATC CCTGTGCAA TGAAGTACC TGTACCACTG TGGCCAACCA GTTCTCCTGC AAATGCCTCA CAGGCTTCAC AGGGCAGAAA
L S H P C A N G S T C T T V A N Q F S C K C L T G F T G Q K>

550 560 570 580 590 600 610 620 630
TGTGAGACTG ATGTCAATGA GTGTGACATT CCAGGACACT GCCAGCATGG TGGCACCTGC CTCAACCTGC CTGGTTCTA CCAGTGCCAG
C E T D V N E C D I P G H C Q H G G T C L N L P G S Y Q C Q>

640 650 660 670 680 690 700 710 720
TGGCTCAGG GCTTACAGG CCACTACTGT GACAGCCTGT ATGTGCCCTG TGCACCTCA CCTTGTGTCA ATGGAGGCAC CTGTGGGCAG
C P Q G F T G Q Y C D S L Y V P C A P S P C V N G G T C R Q>

730 740 750 760 770 780 790 800 810
ACTGGTGACT TCACTTTTGA GTGCAACTGC CTTCCAGGT TTGAAGGGAG CACCTGTGAG AGGAATATTG ATGACTGCCC TAACCACAGG
T G D F T F E C N C L P G F E G S T C E R N I D D C P N H R>

FIG.17A

58/68

820 830 840 850 860 870 880 890 900
TGTCAGAATG GAGGGGTTG TGTGGATGGG GTCAACACTT ACAACTGCCG CTGTCCCCCA CAATGGACAG GACAGTTCTG CACAGAGGAT
C Q N G G V C V D G V N T Y N C R C P P Q W T G Q F C T E D>

910 920 930 940 950 960 970 980 990
GTGGATGAAT GCCTGCTGCA GCCCAATGCC TGTCAAAATG GGGGCACCTG TGCCAACCCG AATGGAGGCT ATGGCTGTGT ATGTGTCAAC
V D E C L L Q P N A C Q N G G T C A N R N G G Y G C V C V N>

1000 1010 1020 1030 1040 1050 1060 1070 1080
GGCTGGAGTG GAGATGACTG CAGTCAGAAC ATTGATGATT GTGCCITGCG CTCCTGTACT CCAGGCTCCA CCTGCATCGA CCGTGTGGCC
G W S G D D C S E N I D D C A F A S C T P G S T C I D R V A>

1090 1100 1110 1120 1130 1140 1150 1160 1170
TCCTTCTCTT GCATGTGCCC AGAGGGGAAG GCAGGTCTCC TGTGTATCT GGATGATGCA TGCATCAGCA ATCCTTGCCA CAAGGGGGCA
S F S C M C P E G K A G L L C H L D D A C I S N P C H K G A>

1180 1190 1200 1210 1220 1230 1240 1250 1260
CTGTGTGACA CCAACCCCT AAATGGGCAA TATATTGCA CCTGCCACA AGGCTACAAA GGGGCTGACT GCACAGAAGA TGTGGATGAA
L C D T N P L N G Q Y I C T C P Q G Y K G A D C T E D V D E>

1270 1280 1290 1300 1310 1320 1330 1340 1350
TGTGCCATGG CCAATAGCAA TCCTTGTGAG CATGCAGGAA AATGTGTGAA CACGGATGGC GCCTTCCACT GTGAGTGTCT GAAGGGTAT
C A M A N S N P C E H A G K C V N T D G A F H C E C L K G Y>

1360 1370 1380 1390 1400 1410 1420 1430 1440
GCAGGACCTC GTTGTGAGAT GGACATCAAT GAGTGCCATT CAGACCCCTG CCAGAATGAT GCTACCTGTC TGGATAAGAT TGGAGGCTTC
A G P R C E M D I N E C H S D P C Q N D A T C L D K I G G F>

1450 1460 1470 1480 1490 1500 1510 1520 1530
ACATGTCTGT GCATGCCAGG TTTCAAAGGT GTCCATTGTG AATTAGAAAT AAATGAATGT CAGAGCAACC CTGTGTGAA CAATGGGCAG
T C L C M P G F K G V H C E L E I N E C Q S N P C V N N G O>

1540 1550 1560 1570 1580 1590 1600 1610 1620
TGTGTGGATA AAGTCAATCG TTTCCAGTGC CTGTGTCTC CTGGTTTAC TGGGCCAGTT TGGCAGATTG ATATTGATGA CTGTTCAGT
C V D K V N R F Q C L C P P G F T G P V C Q I D I D D C S S>

FIG.17B

SUBSTITUTE SHEET (RULE 26)

59/68

1630 1640 1650 1660 1670 1680 1690 1700 1710
* * * * *
ACTCCGTGTC TGAATGGGCG AAAGTGTATC GATCACCCTGA ATGGCTATGA ATGCCAGTGT GCCACAGGTT TCACTGGTGT GTTGTGTGAG
T P C L N G A K C I D H P N G Y E C Q C A T G F T G V L C E>

1720 1730 1740 1750 1760 1770 1780 1790 1800
* * * * *
GAGAACATTG ACAACTGTGA CCCCGATCCT TGCCACCATG GTCAGTGTCA GGATGGTATT GATTCTTACA CCTGCATCTG CAATCCCGGG
E N I D N C D P D P C H H G Q C Q D G I D S Y T C I C N P G>

1810 1820 1830 1840 1850 1860 1870 1880 1890
* * * * *
TACATGGGCG CCATCTGCAG TGACCAGATT GATGAATGTT ACAGCAGCCC TTGCCTGAAC GATGGTGGCT GCATTGACCT GGTCAATGGC
Y M G A I C S D Q I D E C Y S S P C L N D G R C I D L V N G>

1900 1910 1920 1930 1940 1950 1960 1970 1980
* * * * *
TACCACTGCA ACTGCCAGCC AGGCACGTCA GGGGTTAATT GTGAAATTAA TTTTGATGAC TGTGCAAGTA ACCCTTGATAT CCATGGAATC
Y Q C N C Q P G T S G V N C E I N F D D C A S N P C I H G I>

1990 2000 2010 2020 2030 2040 2050 2060 2070
* * * * *
TGTATGGATG GCATTAATCG CTACACTTGT GTCTGCTCAC CAGGATTAC AGGCAGAGA TGTAACTTG ACATTGATGA GTGTGCCTCC
C M D G I N R Y S C V C S P G F T G Q R C N I D I D E C A S>

2080 2090 2100 2110 2120 2130 2140 2150 2160
* * * * *
AATCCCTGTC GCAAGGGTGC AACATGTATC AACGGTGTGA ATGGTTTCCG CTGTATATGC CCCGAGGAC CCCATCACCC CAGCTGCTAC
N P C R K G A T C I N G V N G F R C I C P E G P H H P S C Y>

2170 2180 2190 2200 2210 2220 2230 2240 2250
* * * * *
TCACAGGTGA ACGAATGCCT GAGCAATCCC TGCATCCATG GAACTGTAC TGGAGGTCTC AGTGGATATA AGTGTCTCTG TGATGCAGGC
S Q V N E C L S N P C I H G N C T G G L S G Y K C L C D A G>

2260 2270 2280 2290 2300 2310 2320 2330 2340
* * * * *
TGGGTTGGCA TCAACTGTGA AGTGGACAAA AATGAATGCC TTTCGAATCC ATGCCAGAAT GGAGGAACCT GTGACAATCT GGGAATGGA
W V G I N C E V D K N E C L S N P C Q N G G T C D N L V N G>

2350 2360 2370 2380 2390 2400 2410 2420 2430
* * * * *
TACAGGTGTA CTTGCAAGAA GGCCTTTAAA GGCTATAACT GCCAGGTGAA TATTGATGAA TGTGCCTCAA ATCCATGCCT GAACCAAGGA
Y R C T C K F G F K G Y N C Q V N I D E C A S N P C L N Q G>

FIG.17C

SUBSTITUTE SHEET (RULE 26)

60/68

2440 2450 2460 2470 2480 2490 2500 2510 2520
ACCTGCTTTG ATGACATAAG TGGCTACACT TGCCACTGTG TGCTGCCATA CACAGGCAAG AATTGTCAGA CAGTATTGGC TCCCTGTTCC
T C F D D I S G Y T C H C V L P Y T G K N C Q T V L A P C S>

2530 2540 2550 2560 2570 2580 2590 2600 2610
CCAAACCCTT GTGAGAATGC TGCTGTTTGC AAAGAGTCAC CAAATTTTGA GAGTTATACT TGCTTGTGTG CTCCTGGCTG GCAAGGTCAG
P N P C E N A A V C K E S P N F E S Y T C L C A P G W Q G Q>

2620 2630 2640 2650 2660 2670 2680 2690 2700
CGGTGTACCA TTGACATTGA CGAGTGTATC TCCAAGCCCT GCATGAACCA TGGTCTCTGC CATAACACCC AGGGCAGCTA CATGTGTGAA
R C T I D I D E C I S K P C M N H G L C H N T Q G S Y M C E>

2710 2720 2730 2740 2750 2760 2770 2780 2790
TGTCCACCAG GCTTCAGTGG TATGGACTGT GAGGAGGACA TTGATGACTG CCTTGCCAAT CCTTGCCAGA ATGGAGGTTG CTGTATGGAT
C P P G F S G M D C E E D I D D C L A N P C Q N G G S C M D>

2800 2810 2820 2830 2840 2850 2860 2870 2880
GGAGTGAATA CTTTCTCCTG CCTCTGCCCT CCGGGTTTCA CTGGGGATAA GTGCCAGACA GACATGAATG AGTGTCTGAG TGAACCTGT
G V N T F S C L C L P G F T G D K C Q T D M N E C L S E P C>

2890 2900 2910 2920 2930 2940 2950 2960 2970
AAGAATGGAG GGACCTGCTC TGACTACGTC AACAGTTACA CTTGCAAGTG CCAGGCAGGA TTTGATGGAG TCCATTGTGA GAACAACATC
K N G G T C S D Y V N S Y T C K C Q A G F D G V H C E N N I>

2980 2990 3000 3010 3020 3030 3040 3050 3060
AATGAGTCCA CTGAGAGCTC CTGTTTCAAT GGTGGCACAT GTGTGATGG GATTAACCTC TTCTCTTGCT TGTGCCCTGT GGGTTTCAC
N E C T E S S C F N G G T C V D G I N S F S C L C P V G F T>

3070 3080 3090 3100 3110 3120 3130 3140 3150
GGATCCTTCT GCCTCCATGA GATCAATGAA TGCAGCTCTC ATCCATGCCT GAATGAGGGA ACGTGTGTTG ATGGCCTGGG TACCTACCGC
G S F C L H E I N E C S S H P C L N E G T C V D G L G T Y R>

3160 3170 3180 3190 3200 3210 3220 3230 3240
TGCAGCTGCC CCCTGGGCTA CACTGGGAAA AACTGTCAGA CCCTGGTGAA TCTCTGCACT CCGTCTCCAT GTAAAAACAA AGGTACTTCT
C S C P L G Y T G K N C O T L V N L C S R S P C K N K G T C>

FIG.17D

SUBSTITUTE SHEET (RULE 26)

61/68

3250 3260 3270 3280 3290 3300 3310 3320 3330
GTTCAAGAAA AAGCAGAGTC CCAGTGCCTA TGTCCATCTG GATGGGCTGG TGCCTATTGT GACGTGCCCC ATGTCTCTTG TGACATAGCA
V Q K K A E S Q C L C P S G W A G A Y C D V P N V S C D I A>

3340 3350 3360 3370 3380 3390 3400 3410 3420
GCCTCCAGGA GAGGTGTGCT TGTGAACAC TTGTGCCAGC ACTCAGGTGT CTGCATCAAT GCTGGCAACA CGCATTACTG TCAGTGGCCC
A S R R G V L V E H L C Q H S G V C I N A G N T H Y C Q C P>

3430 3440 3450 3460 3470 3480 3490 3500 3510
CTGGGCTATA CTGGGAGCTA CTGTGAGGAG CAACTCGATG AGTGTGGCTG CAACCCCTGC CAGCACGGGG CAACATGCAG TGACTTCATT
L G Y T G S Y C E E Q L D E C A S N P C Q H G A T C S D F I>

3520 3530 3540 3550 3560 3570 3580 3590 3600
GGTGGATACA GATGGGAGTG TGTCCCAGGC TATCAGGGTG TCAACTGTGA GTATGAAGTG GATGAGTGGC AGAATCAGCC CTGCCAGAAT
G G Y R C E C V P G Y Q G V N C E Y E V D E C Q N Q P C Q N>

3610 3620 3630 3640 3650 3660 3670 3680 3690
GGAGGCACCT GTATTGACCT TGTGAACCAT TTCAAGTGCT CTTGCCCAACC AGGCACTCGG GGCTACTCT GTGAAGAGAA CATTGATGAC
G G T C I D L V N H F K C S C P P G T R G L L C E E N I D D>

3700 3710 3720 3730 3740 3750 3760 3770 3780
TGTGCCCCGG GTCCCCATTG CCTTAATGGT GGTCAAGTGA TGGATAGGAT TGGAGGCTAC AGTTGTGGCT GCTTGCCCTGG CTTTGCTGGG
C A R G P H C L N G G Q C M D R I G G Y S C R C L P G F A G>

3790 3800 3810 3820 3830 3840 3850 3860 3870
GAGCGTTGTG AGGGAGACAT CAACGAGTGC CTCTCCAACC CCTGCAGCTC TGAGGGCAGC CTGGACTGTA TACAGCTCAC CAATGACTAC
E R C E G D I N E C L S N P C S S E G S L D C I Q L T N D Y>

3880 3890 3900 3910 3920 3930 3940 3950 3960
CTGTGTGTTT GCGTAGTGC CTTTACTGGC CGGCACTGTG AAACCTTCGT CGATGTGTGT CCCCAGATGC CCTGCCCTGAA TGGAGGGACT
L C V C R S A F T G R H C E T F V D V C P Q M P C L N G G T>

3970 3980 3990 4000 4010 4020 4030 4040 4050
TGTGCTGTGG CCAGTAACAT GCGTATGGT TTCATTGGC GTGTGCCCC GGGATTTTCC GGGCAAGGT GCCAGAGCAG CTGTGGACAA
C A V A S N M P D G F I C R C P P G F S G A R C Q S S C G Q>

FIG.17E

62/68

4060 4070 4080 4090 4100 4110 4120 4130 4140
GTGAAATGTA GGAAGGGGGA GCAGTGTGTG CACACCGCCT CTGGACCCCG CTGCTTCTGC CCCAGTCCCC GGGACTGCGA GTCAGGCTGT
V K C R K G E Q C V H T A S G P R C F C P S P R D C E S G C>

4150 4160 4170 4180 4190 4200 4210 4220 4230
GCCAGTAGCC CCTGCCAGCA CGGGGGCAGC TGCCACCCTC AGCGCCAGCC TCCTTATTAC TCCTGCCAGT GTGCCCCACC ATTCTCGGT
A S S P C Q H G G S C H P Q R Q P P Y Y S C Q C A P P F S C>

4240 4250 4260 4270 4280 4290 4300 4310 4320
AGCCGCTGTG AACTCTACAC GGCACCCCCC AGCACCCCTC CTGCCACCTG TCTGAGCCAG TATTGTGCCG ACAAAGCTCG GGATGGCGTC
S R C E L Y T A P P S T P P A T C L S Q Y C A D K A R D G V>

4330 4340 4350 4360 4370 4380 4390 4400 4410
TGTGATGAGG CCTGCAACAG CCATGCCTGC CAGTGGGATG GGGGTGACTG TTCTCTCACC ATGGAGAACC CCTGGGCCAA CTGCTCCTCC
C D E A C N S H A C Q W D G G D C S L T M E N P W A N C S S>

4420 4430 4440 4450 4460 4470 4480 4490 4500
CCACTTCCTT GCTGGGATTA TATCAACAAC CAGTGTGATG AGCTGTGCAA CACGGTCGAG TGCCTGTTTG ACAACTTTGA ATGCCAGGG
P L P C W D Y I N N Q C D E L C N T V E C L F D N F E C Q G>

4510 4520 4530 4540 4550 4560 4570 4580 4590
AACAGCAAGA CATGCAAGTA TGACAAATAC TGTCCAGACC ACTTCAAAGA CAACCACTGT AACCAGGGGT GCAACAGTGA GGAGTGTGGT
N S K T C K Y D K Y C A D H F K D N H C N Q G C N S E E C G>

4600 4610 4620 4630 4640 4650 4660 4670 4680
TGGGATGGGC TGGACTGTGC TGCTGACCAA CCTGAGAACC TGGCAGAAGG TACCCTGGTT ATTGTGGTAT TGATGCCACC TGAACAACG
W D G L D C A A D Q P E N L A E G T L V I V V L M P P E Q L>

4690 4700 4710 4720 4730 4740 4750 4760 4770
CTCCAGGATG CTGCGAGCTT CTGCGGGCA CTGGGTACCC TGCTCCACAC CAACCTGCCG ATTAAGCGGG ACTCCAGGG GGAACICATG
L Q D A R S F L R A L G T L L H T N L R I K R D S Q G E L M>

4780 4790 4800 4810 4820 4830 4840 4850 4860
GTGTACCCCT ATTATGGTGA GAAGTCAGCT GCTATGAAGA AACAGAGGAT GACACGCAGA TCCCTTCCTG GTGAACAAGA ACAGGAGGTG
V Y P Y Y G E K S A A M K K Q R M T R R S L P G E Q E Q E V>

FIG.17F

SUBSTITUTE SHEET (RULE 26)

63/68

4870 4880 4890 4900 4910 4920 4930 4940 4950
GCTGGCTCTA AAGTCTTTCT GGAAATTGAC AACCGCCAGT GTGTTCAGA CTCAGACCAC TGCTTCAAGA ACACGGATGC AGCAGCAGCT
A G S K V F L E I D N R Q C V Q D S D H C F K N T D A A A A>

4960 4970 4980 4990 5000 5010 5020 5030 5040
CTCTGGCCT CTCAGCCAT ACAGGGGACC CTGTCATACC CTCTGTGTC TGTCGTGAGT GAATCCCTGA CTCCAGAACC CACTCAGCTC
L L A S H A I Q G T L S Y P L V S V V S E S L T P E R T Q L>

5050 5060 5070 5080 5090 5100 5110 5120 5130
CTCTATCTCC TTGCTGTGC TGTGTGATC ATTCTGTTA TTATCTGCT GCGGTAATC ATGGCAAAAC GAAAGCGTAA GCATGGCTCT
L Y L L A V A V V I I L F I I L L G V I M A K R K R K H G S>

5140 5150 5160 5170 5180 5190 5200 5210 5220
CTCTGGCTGC CTGAAGGTT CACTCTTGC CGAGATGCA GCAATCACA CCGTCGTGAG CCAGTGGGAC AGGATGCTGT GCGGCTGAAA
L W L P E G F T L R R D A S N H K R R E P V G Q D A V G L K>

5230 5240 5250 5260 5270 5280 5290 5300 5310
AATCTCTCAG TGCAAGTCTC AGAAGCTAAC CTAATTGGTA CTGGAACAAG TGAACACTGG GTGGATGATG AAGGGCCCCA GCCAAAGAAA
N L S V Q V S E A N L I G T G T S E H W V D D E G P Q P K K>

5320 5330 5340 5350 5360 5370 5380 5390 5400
GTAAAGCTG AAGATGAGGC CTTACTCTCA GAAGAAGATG ACCCCATTGA TCGACGGCCA TGGACACAGC AGCACCTTGA AGCTGCAGAC
V K A E D E A L L S E E D D P I D R R P W T Q Q H L E A A D>

5410 5420 5430 5440 5450 5460 5470 5480 5490
ATCCGTAGGA CACCATCGCT GGCTCTCACC CCTCCTCAG CAGAGCAGGA GGTGGATGTG TTAGATGTGA ATGTCCTGG CCCAGATGGC
I R R T P S L A L T P P Q A E Q E V D V L D V N V R G P D G>

5500 5510 5520 5530 5540 5550 5560 5570 5580
TGCACCCCAT TGATGTGGC TTCTCTCCGA GGAGGCAGCT CAGATTGAG TGATGAAGAT GAAGATGCAG AGGACTCTTC TGCTAACATC
C T P L M L A S L R G G S S D L S D E D E D A E D S S A N I>

5590 5600 5610 5620 5630 5640 5650 5660 5670
ATCAGAGACT TGGTCTACCA GGGTGCCAGC CTCCAGGCC AGACAGACCG GACTGGTGAG ATGGCCCTGC ACCTTGACAG CCGCTACTCA
I T D L V Y Q G A S L Q A Q T D R T G E M A L H L A A R Y S>

FIG.17G

SUBSTITUTE SHEET (RULE 26)

64/68

5680 5690 5700 5710 5720 5730 5740 5750 5760
* * * * *
CGGCTGATG CTGCCAAGCG TCTCCTGGAT GCAGGTGCAG ATGCCAATGC CCAGGACAAC ATGGGCGGCT GTCCACTCCA TCCTGCAGTC
R A D A A K R L L D A G A D A N A Q D N M G R C P L H A A V>

5770 5780 5790 5800 5810 5820 5830 5840 5850
* * * * *
GCAGCTGATG CCCAAGGTGT CTTCCAGATT CTGATTGCA ACCGAGTAAC TGATCTAGAT GCCAGGATGA ATGATGGTAC TACACCCCTG
A A D A Q G V F Q I L I R N R V T D L D A R M N D G T T P L>

5860 5870 5880 5890 5900 5910 5920 5930 5940
* * * * *
ATCCTGGCTG CCCGCTGGC TGTGGAGGGA ATGGTGGCAG AACTGATCAA CTGCCAAGCG GATGTGAATG CAGTGGATGA CCATGGAAAA
I L A A R L A V E G M V A E L I N C Q A D V N A V D D H G K>

5950 5960 5970 5980 5990 6000 6010 6020 6030
* * * * *
TCTGCTCTTC ACTGGGCAGC TGCTGTCAAT AATGTGGAGG CAACTCTTTT GTTGTGAAA AATGGGGCCA ACCGAGACAT GCAGGACAAC
S A L H W A A A V N N V E A T L L L L K N G A N R D M Q D N>

6040 6050 6060 6070 6080 6090 6100 6110 6120
* * * * *
AAGGAAGAGA CACCTCTGTT TCTTGCTGCC CGGGAGGGGA GCTATGAAGC AGCCAAGATC CTGTTAGACC ATTTTGCCAA TCGAGACATC
K E E T P L F L A A R E G S Y E A A K I L L D H F A N R D I>

6130 6140 6150 6160 6170 6180 6190 6200 6210
* * * * *
ACAGACCATA TGGATCGTCT TCCCGGGAT GTGGCTCGGG ATCGCATGCA CCATGACATT GTGGCCCTTC TGGATGAATA CAATGTGACC
T D H M D R L P R D V A R D R M H H D I V R L L D E Y N V T>

6220 6230 6240 6250 6260 6270 6280 6290 6300
* * * * *
CCAAGCCCTC CAGGCACCGT GTTGAATTCT GCTCTCTCAC CTGTGATCTG TGGGCCCAAC AGATCTTTCC TCAGCCTGAA GCACACCCCA
P S P P G T V L T S A L S P V I C G P N R S F L S L K H T P>

6310 6320 6340 6350 6360 6370 6380 6390 6400
* * * * *
ATGGGCAAGA AGTCTAGACG GCCCAGTCCC AAGAGTACCA TGCCTACTAG CCTCCCTAAC CTTGCCAAGG AGGCAAAGGA TGCCAAGGGT
M G K K S R R P S A K S T M P T S L P N L A K E A K D A K G>

6400 6410 6420 6430 6440 6450 6460 6470 6480
* * * * *
AGTAGGACGA AGAAGTCTCT GAGTGAGAAG GTCCAACGTG CTGAGAGTTC AGTAACTTTA TCCCCTGTTG ATTCCCTAGA ATCTCCTCAC
S R R K K S L S E K V Q L S E S S V T L S P V D S L E S P H>

FIG.17H

SUBSTITUTE SHEET (RULE 26)

65/68

6490 6500 6510 6520 6530 6540 6550 6560 6570
ACGTATGTTT CCGACACCAC ATCCTCTCCA ATGATTACAT CCCCTGGGAT CTTACAGGCC TCACCCAACC CTATGTGGC CACTGCCGCT
T Y V S D T T S S P M I T S P G I L Q A S P N P M L A T A A>

6580 6590 6600 6610 6620 6630 6640 6650 6660
CCTCCTGCCC CAGTCCATGC CCACCATGCA CTATCTTTT CTAACCTTCA TGAATGCAG CCTTTGGCAC ATGGGGCCAG CACTGTGCTT
P P A P V H A Q H A L S F S N L H E M Q P L A H G A S T V L>

6670 6680 6690 6700 6710 6720 6730 6740 6750
CCCTCAGTGA GCCAGTTGCT ATCCCACCAC CACATTGTGT CTCAGGCAG TGGCAGTGCT GGAAGCTTGA GTAGGCTCCA TCCAGTCCCA
P S V S Q L L S H H H I V S P G S G S A G S L S R L H P V P>

6760 6770 6780 6790 6800 6810 6820 6830 6840
GTCCCAGCAG ATGGATGAA CCGCATGGAG GTGAATGAGA CCCAGTACAA TGACATGTTT GGTATGGTCC TGGCTCCAGC TGAGGGCACC
V P A D W M N R M E V N E T Q Y N E M F G M V L A P A E G T>

6850 6860 6870 6880 6890 6900 6910 6920 6930
CATCCTGGCA TAGCTCCCCA GAGCAGGCCA CCTGAAGGGA AGCACATAAC CACCCCTCGG GAGCCCTTGC CCCCATTGT GACTTCCAG
H P G I A P Q S R P P E G K H I T T P R E P L P P I V T F Q>

6940 6950 6960 6970 6980 6990 7000 7010 7020
CTCATCCCTA AAGGCAGTAT TGCCCAACCA GGGGGGGCTC CCCAGCCTCA GTCCACCTGC CCTCCAGCTG TTGGGGGCCC CCTGCCCACC
L I P K G S I A Q P A G A P Q P Q S T C P P A V A G P L P T>

7030 7040 7050 7060 7070 7080 7090 7100 7110
ATGTACCAGA TTCCAGAAAT GGCCCGTTTG CCCAGTGTGG CTTTCCCCAC TGCCATGATG CCCCAGCAGG ACGGGCAGGT AGCTCAGACC
M Y Q I P E M A R L P S V A F P T A M M P Q Q D G Q V A Q T>

7120 7130 7140 7150 7160 7170 7180 7190 7200
ATTCTCCCAG CCTATCATCC TTTCCCAGCC TCTGTGGGCA AGTACCCAC ACCCCCTTCA CAGCACAGTT ATGCTTCCTC AAATGCTGCT
I L P A Y H P F P A S V G K Y P T P P S Q H S Y A S S N A A>

7210 7220 7230 7240 7250 7260 7270 7280 7290
GAGCGAACAC CCAGTCACAG TGGTCACCTC CAGGGTGAGC ATCCCTACCT GACACCATCC CCAGAGTCTC CTGACCAGTG GTCAAGTICA
E R I P S H S G H L Q G E H P Y L T P S P E S P D Q W S S S>

FIG.171

SUBSTITUTE SHEET (RULE 26)

66/68

7300 7310 7320 7330 7340 7350 7360 7370 7380
* * * * *
TCACCCCACT CTGCTTCTGA CTGGTCAGAT GTGACCACCA GCCCTACCCC TGGGGGTGCT GGAGGAGGTC AGCGGGGACC TGGGACACAC
S P H S A S D W S D V T T S P T P G G A G G G Q R G P G T H>

7390 7400 7410 7420 7430 7440 7450 7460 7470
* * * * *
ATGTCTGAGC CACCACACAA CAACATGCAG GTTTATGGT GAGAGAGTCC ACCTCCAGTG TAGAGACATA ACTGACTTTT GTAAATGCTG
M S E P P H N N M Q V Y A>

7480 7490 7500 7510 7520 7530 7540 7550 7560
* * * * *
CTGAGGAACA AATGAAGGTC ATCCGGGAGA GAAATGAAGA AATCTCTGGA GCCAGCTTCT AGAGGTAGGA AAGAGAAGAT GTTCTTATTC

7570 7580 7590 7600 7610 7620 7630 7640 7650
* * * * *
AGATAATGCA AGAGAAGCAA TTCGTCAGTT TCACTGGGTA TCTGCAAGGC TTATTGATT TTTAATCTA ATAAGACAAG TTTGTGGAAA

7660 7670 7680 7690 7700 7710 7720 7730 7740
* * * * *
TGCAAGATGA ATACAAGCCT TGGTCCATG TTTACTCTCT TCTATTGGA GAATAAGATG GATGCTTATT GAAGCCCAGA CATTCTTGCA

7750 7760 7770 7780 7790 7800 7810 7820 7830
* * * * *
GCTTGGACTG CATTTTAAGC CCTGCAGGCT TCTGCCATAT CCATGAGAAG ATTCTACACT AGCGTCTGT TGGGAATTAT GCCCTGGAAT

7840 7850 7860 7870 7880 7890 7900 7910 7920
* * * * *
TCTGCCTGAA TTGACCTACG CATCTCCTCC TCCTTGACA TTCTTTTGTG TTCATTGGT GCTTTTGGTT TTGCACCTCT CCGTGATTGT

7930 7940 7950 7960 7970 7980 7990 8000 8010
* * * * *
AGCCCTACCA GCATGTTATA GGGCAAGACC TTTGTGCTTT TGATCATTCT GGCCCATGAA AGCAACTTTG GTCTCCTTTC CCCTCCTGTC

8020 8030 8040 8050 8060 8070 8080 8090 8100
* * * * *
TTCCCGGTAT CCCTTGGAGT CTCACAAGGT TTACTTTGGT ATGGTTCTCA GCACAAACCT TTCAAGTATG TTGTTTCTTT GGAAAATGGA

8110 8120 8130 8140 8150 8160 8170 8180 8190
* * * * *
CATACTGTAT TGTGTTCTCC TGCATATATC ATTCTTGAG AGAGAAGGGG AGAAGAATAC TTTTCTTCAA CAAATTTTGG GGGCAGGAGA

8200 8210 8220 8230 8240 8250 8260 8270 8280
* * * * *
TCCCTTCAAG AGGCTGCACC TTAATTTTTC TTGTCGTGT GCAGGTCTTC ATATAAACTT TACCAGGAAG AAGGGTGTGA GTTTGTGTGT

FIG.17J

SUBSTITUTE SHEET (RULE 26)

67/68

8290 8300 8310 8320 8330 8340 8350 8360 8370
TTTCTGTGTA TGGCCCTGGT CAGTGTAAG TTTTATCCTT GATAGTCTAG TTACTATGAC CCTCCCCACT TTTTAAAC CAGAAAAAGG

8380 8390 8400 8410 8420 8430 8440 8450 8460
TTTGAATGT TGAATGACC AAGAGACAAG TTAACCTGTG CAAGAGCCAG TTACCCACCC ACAGGTCCCC CTACTTCCTG CCAAGCATTG

8470 8480 8490 8500 8510 8520 8530 8540 8550
CATTGACTGC CTGTATGGAA CACATTTGTC CCAGATCTGA GCATTCTAGG CCTGTTTCAC TCCTCACC ACATATGAA ACTAGTCTTA

8560 8570 8580 8590 8600 8610 8620 8630 8640
ACTGTTGAGC CTTTCCTTTC ATATCCACAG AAGACACTGT CTCAAATGTT GTACCTTGC CATTAGGAC TGAACCTTCC TTAGCCCAAG

8650 8660 8670 8680 8690 8700 8710 8720 8730
GGACCCAGTG ACAGTTGTCT TCCGTTTGTG AGATGATCAG TCCTACTGA TTATCTTGTG GTTAAAGGC CTGCTACCA ATCTTCTTT

8740 8750 8760 8770 8780 8790 8800 8810 8820
CACACCGTGT GGTCCGTGTT ACTGGTATAC CCAGTATGTT CTCCTGAAG ACATGGACTT TATATGTTCA AGTGCAGGAA TTGAAAAGTT

8830 8840 8850 8860 8870 8880 8890 8900 8910
GGACTTGTTT TCTATGATCC AAAACAGCCC TATAAGAAGG TTGAAAAGG AGGAAGTATA TAGCAGCCTT TGCTATTTTC TGCTACCAT

8920 8930 8940 8950 8960 8970 8980 8990 9000
TCCTTCTCTC TGAAGCGGCC ATGACATTCC CTTTGGCAAC TAACGTAGAA ACTCAACAGA ACATTTTCTT TTCTAGAGT CACCTTTTAC

9010 9020 9030 9040 9050 9060 9070 9080 9090
ATGATAATGG ACAACTATAG ACTTGTCTAT TGTTGAGACT GATTGCCCCC CACCTGAATC CACTCTCTGT ATTCATGCTC TTGGCAATTT

9100 9110 9120 9130 9140 9150 9160 9170 9180
CTTTGACTTT CTTTAAAGG CAGAAGCATT TTAGTTAATT GTAGATAAG AATAGTTTTC TTCTCTTCT CCTTGGGCA GTTAATAATT

9190 9200 9210 9220 9230 9240 9250 9260 9270
GGTCCATGGC TAACTGCAA CTCCGTCCA GTGCTGTGAT GCCCATGACA CCTGCAAAT AAGTCTGCC TGGCATTTT GTAGATATTA

FIG.17K

68/68

9280	9290	9300	9310	9320	9330	9340	9350	9360
ACAGGTGAAT	TCCCGACTCT	TTTGGTTTGA	ATGACAGTTC	TCATTCCTTC	TATGGCTGCA	AGTATGCATC	AGTGCTTCCC	ACTTACCTGA
9370	9380	9390	9400	9410	9420	9430	9440	9450
TTTGTCGTG	GGTGGCCCCA	TATGGAACC	CTGGGTGTCT	GTTGGCATAA	TAGTTTACAA	ATGGTTTTTT	CAGTCTATC	CAAATTTATT
9460	9470	9480	9490	9500	9510	9520	9530	9540
GAACCAACAA	AAATAATTAC	TTCGGCCCTG	AGATAAGCAG	ATTAAGTTTG	TTCATTCTCT	GCTTTATTCT	CTCCATGTGG	CAACATTCTG
9550	9560	9570	9580	9590	9600	9610	9620	9630
TCAGCCTCTT	TCATAGTGTG	CAAACATTTT	ATCATTCTAA	ATGGTGACTC	TCGCCCCTTG	GACCCATTTA	TTATTCACAG	ATGGGGAGAA
9640	9650	9660	9670	9680	9690	9700	9710	9720
CCTATCTGCA	TGGACCCTCA	CCATCCTCTG	TCCAGCACAC	ACAGTGCAGG	GAGCCAGTGG	CGATGGCGAT	GACTTTCTTC	CCCTGGGAAT

TCC

FIG.17L

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09338

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : 424/85.8; 435/6; 514/1, 2, 24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.8; 435/6; 514/1, 2, 24

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,115,096 (SHOYAB ET AL) 19 MAY 1992, see column 8, lines 12-27, column 11, lines 39-63, column 14, lines 16-68, columns 16-18, column 28, Table IV, and Figure 13.	1-18, 22-28, 31, 34-40, 42-52, 55-58, 63-67, 75-94
A	US, A, 5,132,212 (KIRSCH ET AL) 21 JULY 1992, see column 7, lines 25-36.	23-31, 45, 55-60, 63-67, 84-90
A,E	US, A, 5,264,557 (SALOMON ET AL) 23 NOVEMBER 1993, see column 1, lines 20-49.	1-18, 22-28, 31, 34-40, 42-52, 55-58, 63-67, 75-94

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
13 December 1993

Date of mailing of the international search report
29 DEC 1993

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09338

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THE NEW BIOLOGIST, Volume 2, No.7, issued July 1990, R.J. Greenspan, "The <u>Notch</u> Gene, Adhesion, And Developmental Fate In The <u>Drosophila</u> Embryo", pages 595-600, see abstract.	1-18,22-28,31,34-40,42-52,55-58,63-67,75-94
A	CELL, Volume 67, issued 15 November 1991, I. Rebay et al, "Specific EGF Repeats of Notch Mediate Interactions with Delta and Serrate: Implications for Notch as a Multifunctional Receptor", pages 687-699, see entire document.	1-94
A,P	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 90, issued May 1993, J.F. De Celis et al, "Genetic and molecular characterization of a Notch mutation in its Delta- and Serrate-binding domain in <u>Drosophila</u> ", pages 4037-4031, see page 4037, column 2, paragraph 2.	21,54
A	EUROPEAN JOURNAL OF BIOCHEMISTRY, Volume 190, issued May 1990, J.A. Campos-Ortega et al, "Molecular analysis of a cellular decision during embryonic development of <u>Drosophila melanogaster</u> : epidermogenesis or neurogenesis", pages 1-10, see section bridging pages 4-5, page 8, column 2, paragraphs 2 and 4.	1-20,22,34-40,42-44,46-53,75-83,91-94
A	Biological Abstracts, Volume 93, No.11, issued 01 June 1992, J. Robbins et al, "Mouse mammary tumor gene int-3: A member of the notch gene family transforms mammary epithelial cells", see page AB-465, abstract no. 122736, J. Virol., 66(4), 2594-2599.	23-28,31,45,55-58,63-67,84-90
A	CELL, Volume 66, issued 23 August 1991, L.W. Ellisen et al, "TAN-1, the Human Homolog of the <u>Drosophila</u> <u>Notch</u> gene, Is Broken by Chromosomal Translocations in T Lymphoblastic Neoplasms", pages 649-661, see pages 657-658.	1-18,22-28,31,34-40,42-52,55-58,63-67,75-94

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Int. l. application No.
PCT/US93/09338

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

A61K 31/00, 31/70, 37/02, 39/44, 39/395; C07H 21/04; G01N 33/53, 33/68

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, CA, INPADOC, JICST-E, MEDLINE, search terms: notch protein or gene product, delta protein or gene product, serrate protein or gene product, disease, disorder, cancer, DNA, nucleic acid, anti-sense, therapy or treatment or pharmaceutical

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

- I. Claims 1-18, 22, 34-40, 42-44, 46-52, 75-83 and 91-94, drawn to pharmaceutical compositions comprising a Notch protein, fragments, chimeras, derivatives or analogs of a Notch protein, methods of treating or preventing malignancy or nervous system disorder, a method of promoting tissue regeneration or repair, and a method of treating a benign dysproliferative disorder, classified in Class 514, subclass 2.
- II. Claims 19, 20 and 53, drawn to a pharmaceutical composition comprising a derivative or analog of a Delta protein and a method of treating or preventing a malignancy, classified in Class 514, subclass 2.
- III. Claims 21 and 54, drawn to a pharmaceutical composition comprising a derivative or analog of a Serrate protein, classified in Class 514, subclass 2.
- IV. Claims 23-28, 31, 45, 55-58, 63-67 and 84-90, drawn to a pharmaceutical composition comprising a nucleic acid encoding a Notch protein, fragments or chimeras of a Notch protein, a method of treating or preventing malignancy comprising administration of nucleic acid encoding a Notch protein, a method of treating a patient with a tumor, and a pharmaceutical composition comprising an isolated oligonucleotide consisting of at least six nucleotides and a recombinant cell, classified in Class 514, subclass 44.
- V. Claims 29 and 59, drawn to a pharmaceutical composition comprising nucleic acid encoding a fragment of a Delta protein, and a method of treating or preventing malignancy comprising administration of nucleic acid encoding a Delta protein, classified in Class 514, subclass 44.
- VI. Claims 30 and 60, drawn to a pharmaceutical composition comprising nucleic acid encoding a fragment of a Serrate protein, and a method of treating or preventing malignancy comprising administration of nucleic acid encoding a Serrate protein, classified in Class 514, subclass 44.
- VII. Claims 32, 33, 41, 61 and 62, drawn to a pharmaceutical composition comprising an antibody and a method of treating or preventing malignancy comprising administration of antibody, classified in Class 424, subclass 85.8.
- VIII. Claim 68-74, drawn to a method of diagnosing a disease, classified in Class 435, subclass 6.